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# **JOURNAL**

**OF THE**

## **ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS**

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**THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS**

# CONTENTS

## PROCEEDINGS OF THE FORTY-FIRST ANNUAL CONVENTION, OCTOBER, 1925.

	PAGE
Obituary on Edwin Fremont Ladd. By H. W. Wiley.....No. 1, iii	
Officers, Committees, Referees, and Associate Referees of the Association of Official Agricultural Chemists for the Year Ending October, 1926.....	1
Members and Visitors Present, 1925 Meeting.....	9
President's Address, The Purposes and Aims of Agricultural Chemical Analysis. By C. A. Browne.....	15

### WEDNESDAY—MORNING SESSION

Report of the Representative at the National Conference on Pharmaceutical Research. By W. O. Emery.....	24
Report of the Committee on Editing Methods of Analysis. By R. E. Doolittle.....	25
Changes in the Official and Tentative Methods of Analysis Made at the Forty- First Annual Convention, October 26-28, 1925.....	27
Report of the Board of Editors. By R. W. Balcom.....	59
Financial Report on Publications. By R. W. Balcom.....	62
Financial Report of the Secretary-Treasurer. By W. W. Skinner.....	64
Report of the Committee on Definitions of Terms and Interpretation of Results on Fertilizers. By H. D. Haskins.....	66
Report of Committee on Revision of Methods of Soil Analysis. By W. H. Mac- Intire.....	69
Report of the Committee on Recommendations of Referees. By R. E. Doolittle.....	69
Report of Sub-Committee A on Recommendations of Referees. By B. B. Ross.....	70
Report of Sub-Committee B on Recommendations of Referees. By H. C. Lythgoe.....	75
Report of Sub-Committee C on Recommendations of Referees. By R. E. Doolittle.....	79

### WEDNESDAY—AFTERNOON SESSION

Report of Committee to Cooperate in Revision of the United States Pharmacopeia. By L. F. Keblor.....	95
Report of the Representatives of the A. O. A. C. on the Board of Governors of the Crop Protection Institute of the National Research Council. By B. L. Hart- well.....	97
Report of the Secretary-Treasurer. By W. W. Skinner.....	98
Report of Committee to Cooperate With Other Committees on Food Definitions. By Julius Hortvet.....	103
Report of Committee on Sampling. By F. C. Blanck.....	107
Report of Committee to Consider the Advisability of Studying Methods for the Analysis of Paint. By W. F. Hand.....	107
Report of Committee on Bibliography. By W. W. Skinner.....	110
Report of Auditing Committee. By H. B. McDonnell.....	111
Report of Nominating Committee. By F. P. Veitch.....	111
Report of Committee on Resolutions. By G. S. Fraps.....	112

### CONTRIBUTED PAPERS

Formaldehyde in Certain Marine Products. By D. B. Dill and P. B. Clark.....	117
The Quantitative Determination of Unsaponifiable Matter in Wheat Flour, Ali- mentary Pastes, and Eggs. By Raymond Hertwig and L. H. Bailey.....	122

Guilford L. Spencer. Tribute by H. W. Wiley.....No. 2, iii	
R. E. Doolittle. Announcement of death.....No. 2, vi	

### MONDAY—MORNING SESSION

Report on Waters, Brine, and Salt. By C. H. Badger.....	125
Report on Insecticides and Fungicides. By J. J. T. Graham.....	127

	PAGE
Report on Reaction Value of Soils. By P. S. Burgess.....	138
Report on Liming Materials. By W. M. Shaw.....	141
Report on Feeding Stuffs. By L. E. Bopst.....	146
Report on the Method for the Determination of Starch in the Presence of Interfering Polysaccharides. By M. R. Coe.....	147
Report on Stock Feed Adulteration. By H. E. Gensler.....	148
Report on Method for Determination of Moisture by Distillation with Toluene. By W. F. Sterling.....	153
Report on Sugars and Sugar Products. By H. S. Paine.....	154
Report on Maple Products. By H. M. Lancaster.....	155
Report on Drying, Densimetric, and Refractometric Methods. By M. Schneller.....	156
Report on Polariscopic Methods. By F. W. Zerban.....	166
Report on Chemical Methods for Reducing Sugars. By R. F. Jackson.....	178
Committees Named by the President.....	180

#### MONDAY—AFTERNOON SESSION

Report on Fertilizers. By G. S. Fraps.....	181
The Gravimetric Determination of Phosphoric Acid. By W. H. Ross.....	182
Report on Nitrogen. By A. L. Prince.....	187
Report on Potash. By A. P. Kerr.....	191
A Method for Potash in Mixed Fertilizers. By G. S. Fraps.....	192
Report on Inorganic Plant Constituents. By A. J. Patten.....	193
Report on Sulfur and Phosphorus in the Seed of Plants. By W. L. Latshaw.....	194
Corrections.....	196

#### CONTRIBUTED PAPERS

Comparison of the Commercial Grading of Barley with the Macroscopical and Chemical Analysis. By L. C. Mitchell.....	197
The Analysis of Butter. By L. C. Mitchell and Samuel Alfend.....	209
The Separation of Formic Acid in Food Products by Distillation with Xylene. By J. K. Morton and G. C. Spencer.....	221
Determination of Strychnine in Poisoned Grains. By J. W. Elmore.....	224

Obituary on Charles Dayton Woods. By J. M. Bartlett.....	No. 3, iii
--	------------

#### MONDAY—AFTERNOON SESSION—Continued

Report on Dairy Products. By Julius Hortvet.....	231
Report on the Determination of Moisture in Cheese. By L. C. Mitchell.....	238
Report on Moisture in Dried Milk. By J. T. Keister.....	243
On the Determination of Casein in Milk. By H. C. Waterman.....	246
Report on Fats and Oils. By G. S. Jamieson.....	247
Report on Baking Powder. By L. H. Bailey.....	253
Report on Fluorides in Baking Powder. By J. K. Morton.....	262

#### DRUG SECTION

Notice of meeting in Brussels, Belgium. By A. G. Dumez.....	266
Report on Drugs. By A. E. Paul.....	266
Report on Acetylsalicylic Acid. By C. W. Harrison.....	278
Report on Determination of Alcohol in Drug Products. By E. V. Lynn.....	282
Report on Arsenicals—Method for the Determination of Arsenic in Sodium Cacodylate. By C. K. Glycart.....	286
Report on Camphor and Monobromated Camphor—The Estimation of Camphor in Pills and Tablets. By E. O. Eaton.....	288
Report on Chaulmoogra Oil. By L. E. Warren.....	290
Report on Chloramine Products. By L. Jones.....	295
Report on Chloroform and Carbon Tetrachloride in Drug Products. By H. O. Moraw.....	296
Report on Ipecac Alkaloids. By A. R. Bliss, Jr.....	301
Report on Laxatives and Bitter Tonics. By H. C. Fuller.....	306
Report on Mercurials. By G. C. Spencer.....	307
Report on Pyramidon. By William Rabak.....	309
Report on the Separation of Quinine and Strychnine. By F. L. Elliott.....	310

# CONTENTS

V

## PAGE

Report on Silver Proteinates. By M. L. Hitchcock.....	312
Report on Nitroglycerin. By A. W. Hanson.....	316
Report on Apomorphine Hydrochloride. By C. K. Glycart.....	323
Report on Santonin. By S. Palkin.....	326
Report on Bioassay of Drugs. By E. W. Schwartz.....	330

## CONTRIBUTED PAPERS

Detection and Determination of Lactic Acid in the Presence of Other Organic Acids. By E. K. Nelson.....	331
Detection of Added Pepper Shells in Pepper. By E. R. Smith, S. Alfend, and L. C. Mitchell.....	333
Method for the Determination of Acidity of Highly Colored Fruit-Type Products. By C. H. Badger and J. W. Sale.....	342

Obituary on Charles Lyndall Penny. By H. H. Hanson..	No. 4, iii
--	------------

## TUESDAY—MORNING SESSION

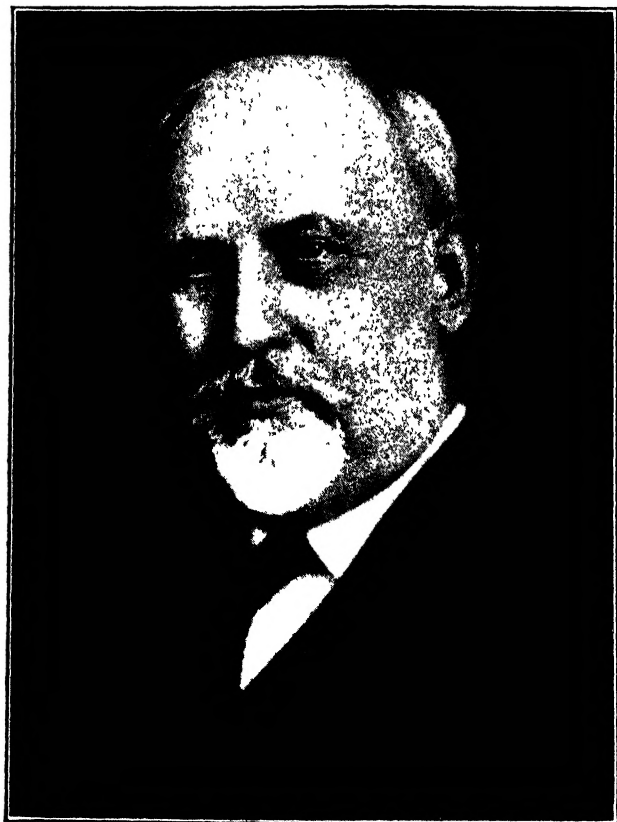
Report on Reagents. By G. C. Spencer.....	347
Report on Eggs and Egg Products. By Raymond Hertwig.....	348
Report on Methods for Determination of Total Solids in Liquid Eggs and Powdered Dried Eggs. By J. C. Palmer.....	354
Report on Food Preservatives. By W. W. Randall.....	358
Report on Coloring Matters in Foods. By C. F. Jablonski.....	362
Report on Metals in Foods. By W. F. Clarke.....	364
Announcement of International Congress of Soil Science. By J. G. Lipman.....	367
Report on Fruits and Fruit Products. By P. L. Gowen.....	367
Report on Added Water in Grape Juice. By B. G. Hartmann.....	374
Report on Fruit Acids. By E. K. Nelson.....	375
Address by Dr. H. W. Wiley.....	382

## TUESDAY—AFTERNOON SESSION

Report on Cereal Foods. By Raymond Hertwig ..	389
Report on Moisture in Flour. By G. C. Spencer ..	404
Report on Ash in Flour. By C. E. Mangels.....	409
Report on Glutenin in Wheat Flour. By M. J. Blish.....	417
Report on Methods for Sampling Flour. By H. Runkel.....	423
Report on Methods for Examination of Bread. By L. H. Bailey.....	426
Report on Fat (by Acid Hydrolysis), Lipoids and Lipoid Phosphoric Acid ( $P_2O_5$ ), and Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol in Flour. By Samuel Alfend.....	429
Report on Hydrogen-ion Concentration of Flour. By C. H. Bailey.....	433
Report on Specific Gravity and Alcohol. By R. M. Hann.....	437
Report on Vinegars. By J. O. Clarke.....	440
Report on Flavors and Non-alcoholic Beverages. By J. W. Sale.....	446
Report on Meat and Meat Products. By R. H. Kerr.....	457
Report on Gelatin. By E. H. Berry.....	458
Address—Diversifying the Uses of Farm Crops. By W. M. Jardine, Secretary of Agriculture.....	460
Report on Cacao Products. By E. M. Bailey.....	461
Report on Turpentine. By V. E. Grotlich.....	473

## CONTRIBUTED PAPERS

Note on the Diethylphthalate Test. By H. Wales.....	476
Iodine Number of Paprika Oil. By L. C. Mitchell.....	477
Note on Influence of Peptic Digestion in Determination of Total Carbohydrates in Cereal Products. By B. G. Hartmann and Fred Hillig.....	482



EDWIN FREMONT LADD, 1859-1925

## A TRIBUTE TO SENATOR LADD.

Edwin Fremont Ladd was born in Starks, Maine, December 13, 1859. He took the degree of B. S. from the University of Maine in 1884, and in 1915 this university conferred upon him an honorary LL.D. degree. He married Miss Rizpah Sprogle, of Annapolis, Maryland, August 16, 1893. He was assistant chemist first, and afterward chief chemist of the New York State Experiment Station, from 1884 to 1890. He was dean of the School of Chemistry and Pharmacy and professor of chemistry of the North Dakota Agricultural College from 1890 to 1916. During that time he also held the office of state chemist. He was appointed president of the North Dakota Agricultural College on February 28, 1916. He was made food commissioner of North Dakota in 1902, and he was editor of the "North Dakota Farmer" from 1899 until his death. He was elected to the United States Senate for the term beginning March 3, 1921, and this term would not have expired until 1927. He was a fellow of the American Association for the Advancement of Science; member of the American Chemical Society; Society of Chemical Industry, of London; Society for the Promotion of Agricultural Science, and past president of the Association of State and National Food and Dairy Departments and of the Association of Official Agricultural Chemists. He was a member of the Standards Committee on Food Products for the United States, after the passage of the pure food law. During the war he was Federal food administrator of North Dakota.

Ladd's activity as a member of the Association of Official Agricultural Chemists began apparently in 1889, at which time he did some work for the association as a collaborator. He became increasingly active as the years passed, serving as a referee on at least two different subjects and as a member of various committees. At the 1913 meeting he was elected president of the association for the ensuing year. It was during the years 1913 and 1914 that the deliberations leading to the establishment of the *Journal of the Association of Official Agricultural Chemists* were held, and Dr. Ladd took an active part in these deliberations. When *The Journal* was established he was appointed a member of its first Board of Editors, and he served in this capacity continuously for about five years. At the meeting in 1921 Senator Ladd, by a unanimous vote, was elected an honorary life member of the association.

It was my good fortune to have known Dr. Ladd personally from the time that he began his work in the New York State Agricultural Experiment Station until the date of his untimely death. He passed away in Baltimore on June 22, 1925. Senator Ladd had strong personal characteristics. He believed in direct action. It did not take him long to make up his mind as to what side of a question he should take; he wanted to know simply which was the right side. If the right side was popular, he was not deterred from espousing it for that reason; if it was unpopular, he seemed to be more eager to uphold the right. In the hearings before the committees that made the regulations for the carrying out of the national pure food law, and before the committees in Congress during the time it was pending, Dr. Ladd was always clear, concise, to the point, and entirely understandable. I remember particularly his attitude in regard to some of the mooted



questions that arose in connection with the enforcement of the Federal food and drugs act. Dr. Ladd was unalterably opposed to the use of any kind of preservatives in food products, except the necessary condimental preservatives. He was particularly antagonistic to the bleaching of flour. When the Secretary of Agriculture had hearings on this question, Dr. Ladd was the most militant, and perhaps the most efficient, witness against the process. I think the arguments, illustrations, and accounts of experiment presented by Dr. Ladd were among the leading factors that led Secretary Wilson to forbid the use of bleaching agents in flour. His opposition to benzoate of soda was equally determined, but it was not so successful with the Federal authorities. He administered the food law of his State rigidly, and he had much to do with its passage. This law represented largely his own views as to what an efficient food administration of a State should be. There were no side entrances or secret passages by means of which the food laws of North Dakota could be circumvented by scheming dealers and manufacturers. When they went to North Dakota with their wares they always found a stone wall which they were unable to scale.

It was due to the popularity attained by Dr. Ladd as chemist, food commissioner and president of the North Dakota Agricultural College that he was induced to enter politics. I think undoubtedly he was the most popular man in North Dakota. The people of his State believed in him; they knew he was absolutely honest and incorruptible. Therefore, he had no difficulty in securing the suffrage of his fellow citizens for the highest honor that the State could offer. When Dr. Ladd became United States Senator he brought to his office an economic program that for the first time in our relationship failed to receive my cordial support. I never asked him to change his views, however, because I knew the man so well I felt convinced that he thought he was entirely right and that it was entirely useless to try to change the views of a man with the firmness of conviction which I knew Senator Ladd held. This, however, abated not a whit my esteem for the man's personality and ability. In the four years of his service in the United States Senate he became, next to La Follette, the most militant progressive in that body. It was the irony of Fate that carried both Senator La Follette and Senator Ladd out of the realm of earthly activities almost at the same time. The two great leaders of the progressive movement died within a short time of each other, Senator Ladd's death occurring just a few days after the death of Senator La Follette.

Senator Ladd's career was not spectacular; it was too earnest to partake of that character. The estimation of his worth as a scientific man, as a State official, as a college president, and as a member of the United States Senate will grow with the succeeding years. To me his death was distinctly personal. I felt that I had lost that friend who had stood by me shoulder to shoulder for thirty years in the fight for pure food legislation and in my efforts to enforce the law regulating the sale of foods and drugs under the terms in which Congress enacted it and with the sole purpose of protecting the people of the United States from harm. In this work I had no more cordial and efficient supporter than Senator Ladd.

H. W. WILEY.

# PROCEEDINGS OF THE FORTY-FIRST ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1925.

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The forty-first annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 26-28, 1925.

The meeting was called to order by the president, C. A. Browne, Bureau of Chemistry, Washington, D. C., on the morning of October 26th, with the following remark: "In opening this forty-first annual convention of the Association of Official Agricultural Chemists, I wish, as a Washingtonian, to extend a very hearty greeting to all the members and to express the hope that your sojourn here in Washington will be pleasant and profitable".

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## OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, FOR THE YEAR ENDING OCTOBER, 1926.

### *Honorary President.*

HARVEY W. WILEY, Mills Building, Washington, D. C.

### *President.*

W. W. RANDALL, Department of Health, Baltimore, Md.

### *Vice-President.*

W. H. MACINTIRE, Agricultural Experiment Station, Knoxville, Tenn.

### *Secretary-Treasurer.*

W. W. SKINNER, Bureau of Chemistry, Washington, D. C.

### *Additional Members of the Executive Committee.*

OSWALD SCHREINER, Washington, D. C.

E. M. BAILEY, New Haven, Conn.



## SPECIAL COMMITTEES.

*Committee on Definitions of Terms and Interpretation of Results on Fertilizers.*H. D. HASKINS (Agricultural Experiment Station, Amherst, Mass.), *Chairman.*

J. W. KELLOGG.

G. S. FRAPS.

C. H. JONES.

R. N. BRACKETT.

*Committee on Revision of Methods of Soil Analysis.*W. H. MACINTIRE (Agricultural Experiment Station, Knoxville, Tenn.), *Chairman.*

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J. A. BIZZELL.

R. STEWART.

A. G. MCCALL.

*Committee on Quartz-Plate Standardization and Normal Weight.*FREDERICK BATES (Bureau of Standards, Washington, D. C.), *Chairman.*

C. A. BROWNE.

F. W. ZERBAN.

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H. J. PATTERSON, College Park, Md.

*Committee on Sampling.*F. C. BLANCK (Bureau of Chemistry, Washington, D. C.), *Chairman.*F. C. BLANCK: *Foods.*J. W. KELLOGG: *Foods and Feeding Stuffs.*A. E. PAUL: *Drugs.*A. G. MCCALL: *Soils and Liming Materials.*C. C. McDONNELL: *Insecticides and Fungicides.*R. N. BRACKETT: *Fertilizers.*F. W. ZERBAN: *Saccharine Products.*R. W. FREY: *Tanning Materials and Leathers.*J. W. SALE: *Water.*A. J. PATTEN: *Plants.**Committee to Consider the Advisability of Studying Methods for the Analysis of Paint.*

W. F. HAND (Agricultural and Mechanical College, Agricultural College, Miss.),

*Chairman.*

W. T. PEARCE.

J. W. KELLOGG.

*Committee on Bibliography.*W. W. SKINNER (Bureau of Chemistry, Washington, D. C.), *Chairman.*

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H. D. HASKINS.

F. P. VEITCH.

W. W. RANDALL.

*Committee on Constitution and By-Laws.*B. B. ROSS (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman.*

E. M. BAILEY.

F. P. VEITCH.

REFEREES AND ASSOCIATE REFEREES.

WATERS, BRINE, AND SALT:

*General referee:* C. H. Badger, Bureau of Chemistry, Washington, D. C.

TANNING MATERIALS AND LEATHERS:

*General referee:* T. D. Jarrell, Bureau of Chemistry, Washington, D. C.

INSECTICIDES AND FUNGICIDES:

*General referee:* J. J. T. Graham, Bureau of Chemistry, Washington, D. C.

SOILS AND LIMING MATERIALS:

*General referee:* W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

REACTION VALUE OF SOILS:

*Associate referee:* P. S. Burgess, Agricultural Experiment Station, Tucson, Ariz.

LIMING MATERIALS:

*Associate referee:* W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

FEEDING STUFFS:

*General referee:* W. F. Sterling, Bureau of Chemistry, Washington, D. C.

STOCK FEED ADULTERATION:

*Associate referee:* H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

MINERAL MIXED FEEDS:

*Associate referee:* H. A. Halvorson, 215 Old Capitol Bldg., St. Paul, Minn.

DETERMINATION OF MOISTURE:

*Associate referee:* F. R. Darkis, Agricultural Experiment Station, College Park, Md.

SUGARS AND SUGAR PRODUCTS:

*General referee:* H. S. Paine, Bureau of Chemistry, Washington, D. C.

MAPLE PRODUCTS:

*Associate referee:* H. M. Lancaster, 317 Queen St., Ottawa, Can.

STARCH CONVERSION PRODUCTS:

*Associate referee:* F. W. Reynolds, Bureau of Chemistry, Washington, D. C.

POLARISCOPIC METHODS:

*Associate referee:* F. W. Zerban, New York, N. Y.

CHEMICAL METHODS FOR REDUCING SUGARS:

*Associate referee:* R. F. Jackson, Bureau of Standards, Washington, D. C.

FERTILIZERS:

*General referee:* G. S. Fraps, College Station, Tex.

PHOSPHORIC ACID:

*Associate referee:* W. H. Ross, Bureau of Soils, Washington, D. C.

NITROGEN:

*Associate referee:* A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

**POTASH:**

*Associate referee:* A. P. Kerr, Agricultural Experiment Station, Baton Rouge, La.

**PLANTS:**

*General referee:* A. J. Patten, Agricultural Experiment Station, E. Lansing, Mich.

**DAIRY PRODUCTS:**

*General referee:* Julius Hortvet, Dairy and Food Department, St. Paul, Minn.

**BUTTER:**

*Associate referee:* L. C. Mitchell, 204 Old Custom House, St. Louis, Mo.

**CHEESE:**

*Associate referee:* E. O. Huebner, Dairy and Food Commission, Madison, Wis.

**MALTED MILK:**

*Associate referee:* B. G. Hartmann, Bureau of Chemistry, Washington, D. C.

**DRIED MILK:**

*Associate referee:* J. K. Keister, Bureau of Chemistry, Washington, D. C.

**ICE CREAM:**

*Associate referee:* A. C. Dahlberg, Agricultural Experiment Station, Geneva, N. Y.

**FATS AND OILS:**

*General referee:* G. S. Jamieson, Bureau of Chemistry, Washington, D. C.

**BAKING POWDERS AND BAKING CHEMICALS:**

*General referee:* L. H. Bailey, Bureau of Chemistry, Washington, D. C.

**DRUGS:**

*General referee:* A. E. Paul, U. S. Food and Drug Inspection Station, 1625 Transportation Bldg., Chicago, Ill.

**ACETYSALICYLIC ACID:**

*Associate referee:* Edward F. Kenney, U. S. Food and Drug Inspection Station, Baltimore, Md.

**ALCOHOL IN DRUGS:**

*Associate referee:* E. V. Lynn, University of Washington, College of Pharmacy, Seattle, Wash.

**ARSENICALS:**

*Associate referee:* H. Wales, Bureau of Chemistry, Washington, D. C.

**COCAINE:**

*Associate referee:* E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif.

**CHAULMOOGRA OIL:**

*Associate referee:* L. E. Warren, Bureau of Chemistry, Washington, D. C.

**CRUDE DRUGS:**

*Associate referee:* J. F. Clevenger, Bureau of Chemistry, Washington, D. C.

**CHLOROFORM AND CHLORAL HYDRATE:**

*Associate referee:* H. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.

**IPECAC ALKALOIDS:**

*Associate referee:* A. R. Bliss, Jr., University of Tennessee, Memphis, Tenn.

**RADIO ACTIVITY IN DRUGS AND WATER:**

*Associate referee:* J. W. Sale, Bureau of Chemistry, Washington, D. C.

**LAXATIVES AND BITTER TONICS:**

*Associate referee:* H. C. Fuller, 2201 New York Ave., Washington, D. C.

**MERCURIALS:**

*Associate referee:* P. W. Morgan, U. S. Food and Drug Inspection Station, Chicago, Ill.

**PYRAMIDON:**

*Associate referee:* Wm. Rabak, U. S. Food and Drug Inspection Station, Minneapolis, Minn.

**MICROCHEMICAL ALKALOID METHODS:**

*Associate referee:* C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.

**SILVER PROTEINATES:**

*Associate referee:* L. Jones, U. S. Food and Drug Inspection Station, Chicago, Ill.

**NITROGLYCERIN:**

*Associate referee:* A. W. Hanson, U. S. Food and Drug Inspection Station, Chicago, Ill.

**TERPIN HYDRATE:**

*Associate referee:* C. W. Harrison, Food and Drug Inspection Station, 218 Water St., Baltimore, Md.

**APOMORPHINE:**

*Associate referee:* A. G. Murray, Bureau of Chemistry, Washington, D. C.

**SANTONIN:**

*Associate referee:* S. Palkin, Bureau of Chemistry, Washington, D. C.

**ETHER:**

*Associate referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

**BIO-ASSAY OF DRUGS:**

*Associate referee:* E. W. Schwartze, Bureau of Chemistry, Washington, D. C.

**TESTING CHEMICAL REAGENTS:**

*General referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

**EGGS AND EGG PRODUCTS:**

*General referee:* Total solids and acidity of lipoids—H. W. Redfield, U. S. Food and Drug Inspection Station, New York, N. Y.

**DETECTION OF DECOMPOSITION:**

*Associate referee:* H. I. Macomber, Bureau of Chemistry, New York, N. Y.

**WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL, UNSAPONIFIABLE MATTER, AND ASH:**

*Associate referee:* J. C. Palmer, U. S. Food and Drug Inspection Station, San Francisco, Calif.

**FOOD PRESERVATIVES:**

*General referee:* W. W. Randall, State Department of Health, Baltimore, Md.

**COLORING MATTERS IN FOODS:**

*General referee:* C. F. Jablonski, U. S. Food and Drug Inspection Station, New York, N. Y.

**METALS IN FOODS:**

*General referee:* W. F. Clarke, Bureau of Chemistry, Washington, D. C.

**ZINC IN DRIED EGGS:**

*Associate referee:* W. E. Kirby, U. S. Food and Drug Inspection Station, New York, N. Y.

**FRUITS AND FRUIT PRODUCTS:**

*General referee:* P. L. Gowen, Bureau of Chemistry, Washington, D. C.

**WATER IN GRAPE JUICE:**

*Associate referee:* B. G. Hartmann, Bureau of Chemistry, Washington, D. C.

**ASH IN FRUIT PRODUCTS:**

*Associate referee:* H. J. Wichmann, U. S. Food and Drug Inspection Station, San Francisco, Calif.

**FRUIT ACIDS:**

*Associate referee:* E. K. Nelson, Bureau of Chemistry, Washington, D. C.

**CANNED FOODS:**

*General referee:* I. L. Miller, State Food and Drug Department, Indianapolis, Ind.

**CEREAL FOODS:**

*General referee:* F. C. Blanck, Bureau of Chemistry, Washington, D. C.

**SAMPLING OF FLOUR:**

*Associate referee:* H. Runkel, U. S. Food and Drug Inspection Station, Minneapolis, Minn.

**MOISTURE IN FLOUR AND IN ALIMENTARY PASTES:**

*Associate referee:* G. C. Spencer, Bureau of Chemistry, Washington, D. C.

**ASH IN FLOUR AND GASOLINE COLOR VALUE:**

*Associate referee:* D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

**GLUTENIN IN FLOUR:**

*Associate referee:* M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

**HYDROGEN-ION CONCENTRATION OF FLOUR:**

*Associate referee:* C. H. Bailey, University of Minnesota, Minneapolis, Minn.

**GLUTEN IN FLOUR:**

*Associate referee:* C. B. Kress, Sperry Flour Mills, Stockton, Calif.

**DIASTATIC VALUE OF FLOUR:**

*Associate referee:* C. O. Swanson, Kansas State Agricultural College, Manhattan, Kans.

**STARCH IN FLOUR:**

*Associate referee:* O. S. Rask, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.

**CHLORINE IN BLEACHED FLOUR:**

*Associate referee:* Armin Scidenberg, Department of Health, New York, N. Y.



**EXPERIMENTAL BAKING TESTS<sup>1</sup>:**

*Associate referee:* M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

**UNSAAPONIFIABLE MATTER AND FAT IN FLOUR AND IN ALIMENTARY PASTES:**

*Associate referee:* Samuel Alfend, U. S. Food and Drug Inspection Station, St Louis, Mo.

**METHODS FOR BREAD ANALYSIS:**

*Associate referee:* L. H. Bailey, Bureau of Chemistry, Washington, D. C.

**SPECIFIC GRAVITY AND ALCOHOL:**

*General referee:* R. M. Hann, Bureau of Chemistry, Washington, D. C.

**VINEGARS:**

*General referee:* J. O. Clarke, U. S. Food and Drug Inspection Station, Savannah, Ga.

**FLAVORS AND NON-ALCOHOLIC BEVERAGES:**

*General referee:* J. W. Sale, Bureau of Chemistry, Washington, D. C.

**MEAT AND MEAT PRODUCTS:**

*General referee:* R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

**SEPARATION OF MEAT PROTEINS:**

*Associate referee:* W. W. Ritchie, Department of Agricultural Chemistry, Columbia, Mo.

**GELATIN:**

*General referee:* E. H. Berry, U. S. Food and Drug Inspection Station, Chicago, Ill.

**SPICES AND OTHER CONDIMENTS:**

*General referee:* W. C. Geagley, State Food and Drug Department, Lansing, Mich.

**CACAO PRODUCTS:**

*General referee:* E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

**MICROSCOPICAL METHODS:**

*Associate referee:* V. A. Pease, Bureau of Chemistry, Washington, D. C.

**CRUDE FIBER:**

*Associate referee:* E. R. Miller, U. S. Food and Drug Inspection Station, New York, N. Y.

**CACAO BUTTER:**

*Associate referee:* L. W. Ferris, U. S. Food and Drug Inspection Station, Buffalo, N. Y.

**NAVAL STORES:**

*General referee:* F. P. Veitch, Bureau of Chemistry, Washington, D. C.

**TURPENTINE:**

*Associate referee:* V. E. Grotlisch, Bureau of Chemistry, Washington, D. C.

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<sup>1</sup> Subject included and appointment made through action of the Executive Committee.

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## PRESIDENT'S ADDRESS.

### THE PURPOSES AND AIMS OF AGRICULTURAL CHEMICAL ANALYSIS<sup>1</sup>.

By C. A. BROWNE (Bureau of Chemistry, Washington, D. C.).

The leading event in the forty-first year of the history of this Association of Official Agricultural Chemists was the publication of a new edition of its "Official and Tentative Methods of Analysis". This volume, the most complete that our association has ever issued, is the embodiment of a vast amount of labor and research, representing as it does the combined activities of many members since the origin of the society in 1884. The comments upon the association's published methods of analysis by chemists outside our membership have usually been favorable. Our first scanty pamphlet of methods, published just forty years ago as Bulletin 7 of the Division of Chemistry, was reproduced by the late Sir William Crookes in the "Chemical News" for January 1, 1886, and the edition just issued has received from foreign reviewers a most laudatory reception. The few words of criticism that have been noted are not carping but are rather expressions of kindly intent which reflect a few of our own misgivings.

The past records of our proceedings show that we have always been cognizant of certain shortcomings. In his presidential address in 1886 Dr. Wiley, in referring to our first bulletin of methods, made this remark: "It would be idle to think that the work so well begun is already perfect. A first step forward has certainly been taken, but the road before us is still a long one". These early words of our honorary president, whom we are happy to have with us again today, apply with even greater force to our latest publication. The methods of official agricultural analysis have enlarged their limits a hundred fold within the last forty years. Fully conscious of even greater extensions in the future we dimly realize how far removed is the goal of perfect attainment.

I wonder sometimes if the founders of our association were aware of what was involved in the coupling of those two words "official" and "agricultural". The original object of this society, as announced in its first constitution, was "to secure as far as possible uniformity in legislation with regard to the regulation of the sale of commercial fertilizers in the different States and uniformity and accuracy in the methods and results of fertilizer analysis". This purpose was the direct outcome of

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<sup>1</sup> Presented Tuesday morning, October 27th, as special order of business for 11 o'clock.



an early regulatory function of our agricultural experiment stations, which was to prevent the deception of farmers in the purchase of commercial fertilizers.

At its fourth annual meeting in 1887 the association felt it necessary to extend its aims so as "to secure uniformity and accuracy in the methods, results and modes of statement of analyses of fertilizers, soils, cattle foods, dairy products and other materials connected with agricultural industry". This new declaration indicates a departure from the exclusively regulatory lines of activity originally laid down, for it includes in its program the analysis of agricultural materials, such as soils, which are not subject to official control. It is interesting to note how at this early stage in the history of our association the interests of some of its members partook less of a regulatory and more of a research character. This tendency has grown with increasing force among the agricultural chemists of America, and it has had a natural reaction upon the work of this association. It is to a consideration of some effects of this influence that I invite your attention this morning.

The celebration at New Haven this month of the fiftieth anniversary of the founding of our first agricultural experiment station has no doubt caused many of you to review the transitions that these institutions have undergone in the course of their development. The influence that the chemists of these stations exercised in their respective States, in organizing an official control of fertilizers, feeding stuffs, and human foods, fills a large part in the history of agricultural commodity regulation. These chemists were an important element in the early membership of our association, and it is unnecessary for me to remind you of their accomplishments. Yet the ultimate aim of the first directors of our agricultural experiment stations was not a regulatory one. According to statements made by some of these officials at the recent New Haven anniversary, the final objective which they kept in view from the very beginning was agricultural research. The latter, however, was too strong a meat for the State politicians who held the purse strings. But the local solons could understand the usefulness of laws that prevented the farmer from being victimized in the purchase of his supplies, and so they readily permitted the experiment stations to derive an income from such regulatory operations. As one of these early directors picturesquely expressed it: "A rapid effective barrage was first directed against the adulteration of fertilizers and cattle feeds in order that the slow-moving batteries of research could be brought into action".

A few officials, however, took the view that commodity inspection was an essential part of experiment station work and, influenced by the successes already achieved, sought to extend the regulatory powers of their stations to a supervision of paints, fabrics, wearing apparel, gasoline, and every other product that is used upon the farm. The late

Senator Ladd, when he was chief chemist of the North Dakota Agricultural Experiment Station, took important steps in this direction, and there is no saying how far this great leader might have gone had he been chosen to decline the call of his State for service in another field. In his presidential address before this association in 1915, Dr. Ladd remarked: "Where we had formerly fertilizer laws, then feeding stuff laws, we now have food laws, drug laws, insecticide and fungicide laws, sanitary laws, and moving rapidly towards us, is the perplexing work with paints, varnishes, textile fabrics, and scores of other products that the official chemist must be prepared to handle". While this association did not follow closely our lamented colleague into the enlarged domain which he pointed out as "a great field, rich for the harvest", we have perhaps gone farther beyond the confines of pure agricultural analysis than some of us imagine.

But in opposition to this idea of extending the regulatory functions of our State experiment stations there soon began to develop a strong countervailing opinion held by many of the first directors—as already indicated—and supported by the practice of European countries, that the undertaking of extensive control work was a serious impediment to agricultural research. Many of the stations began gradually to transfer their regulatory activities to State laboratories in order that more attention might be given to the operations of research and extension. With this partial severance of regulatory from other activities our experiment station chemists applied themselves more diligently to chemical analysis as it concerned the investigations of plant and animal life than to chemical analysis as a means of detecting adulteration. Indeed so great was this reaction among some chemists that a former president of our association, and a well-known authority upon food and drug inspection, recently remarked to me that the detective side of agricultural analysis had been greatly overstressed. In his opinion future books upon the subject must give vastly more attention to chemical analysis as a weapon of agricultural research. Without minimizing to the slightest degree the necessity of strengthening the arms of law enforcement with every possible aid which chemistry can offer, we must admit that the works upon agricultural analysis, our own recent publication included, give proportionately too little attention to methods of agricultural research. Over 95 per cent of the total products of agriculture and industry are genuine, and if the world were entirely free from the misdemeanors of adulteration and false labeling the need for the agricultural analyst would remain as potent as ever.

The work of our association has naturally been influenced by both of these streams of tendency—the attention of its referees being given now to methods of official control and now to methods of agricultural research. Opinions will differ as to how successfully we have adhered to

the safe middle course prescribed by Dr. Wiley's favorite Latin motto: "*In mediis tutissimus ibis*". In a recent review of the new edition of our *Methods of Analysis* by that well-known English publication, "Chemistry and Industry", the statement is made that "out of the 500 odd pages of which the work consists, perhaps only 120 comprise such sections as fall usually within the scope of the agricultural analyst in particular". This estimate might indicate that we have strayed too far from the concerns of husbandry, were it not for the fact that opinions differ so greatly as to where the line should be drawn between agricultural chemistry and other branches of the science. No field of applied chemistry is so broad in its scope or so comprehensive in its bearings as that which pertains to agriculture. It includes not only much of chemistry, but it touches also upon mineralogy, physics, meteorology, plant and animal physiology, mycology, and other correlated sciences. Of the thirty divisions of chemistry into which the subject matter of "Chemical Abstracts" is divided, fifteen have a relationship to agriculture that is immediate and fifteen a relationship that is indirect. In my opinion this association, in the products for which it has established methods of analysis, has adhered in large part to agricultural lines. I believe, however, that our methods for agricultural chemical research require greatly to be strengthened. A few illustrations of where improvement might be made are offered in this connection.

The subject of soils, as previously indicated, was one of the first for which this association proposed methods of analysis that were purely of an investigative character. Yet our society of agricultural chemists has never given to soils the degree of attention that this subject deserves. The review by "Chemistry and Industry" previously quoted very justly reminds us that in the chapter on soils there is no mention of methods of mechanical analysis and that the reader might look in vain for notices of work emanating from such a world-known center as the Rothamstead Experiment Station. In view of the great amount of attention being given at present by agricultural chemists to the hydrogen-ion concentration of soils and its relationship to plant growth and plant pathology, it is highly desirable that the methods of performing this measurement be more adequately treated in forthcoming editions of our book. The same is also true of methods for determining hydrogen-ion concentration in plant and animal fluids, extracts, and other miscellaneous solutions.

The improvement of our methods for determining minute quantities of certain elementary constituents of foods is another desideratum. The needs in our diet of traces of fluorine for tooth formation, of iodine for the prevention of goiter, of iron for the production of hemoglobin, and of other less common elements for specific physiological uses, call for a greater improvement in many of our analytical processes. Indeed, the agricultural products of certain sections of our country are sometimes

advertised as superior because of their higher iodine content and thus, as so often happens, a subject of scientific interest assumes forthwith a regulatory aspect. The reputed beneficial effect of minute quantities of certain substances, such as boron, zinc, and arsenic, as stimulators of development and the surmised injurious effects of traces of mineral and organic poisons as promoters of malignant growths, in plants and animals, are additional reasons for our devoting more attention to analytical refinements.

Attention might be called to the inadequacy of our present methods for determining chlorine in plant and animal products. As is well known, a considerable loss of this element occurs even under the most careful methods of incineration. In order to prevent the escape of liberated chlorine compounds during combustion our official methods recommend the admixture of chemically pure sodium carbonate as a combining agent. But those who have experimented in this field know that this precaution is not always completely effective. Drost<sup>1</sup>, for example, reports a loss of 10 per cent of the total chlorine in the dried residue of milk during incineration with sodium carbonate. The need for greater accuracy of chlorine determinations in plant and animal products is of increasing importance for both regulatory and investigative reasons. Variations in chlorine content of a few hundredths of a per cent have decided lawsuits as to whether agricultural products were damaged by sea water or fresh water; minute variations in chlorine percentage have also decided important sanitary questions such as that of determining whether a suspected milk did or did not contain a pathological secretion.

This association can render increasing service to the chemists who are occupied with agricultural chemical research by making a study and selection of suitable analytical methods for ascertaining the rarer organic constituents of plant and animal substances. Processes are constantly needed for determining glucosides, alkaloids, amino compounds, alcohols, aldehydes, acids, and the innumerable other organic constituents of agricultural products. The association has already made an entrance into this field, and it is hoped that our referees may carry the work onward, for much still remains to be accomplished. When we consider the nutritional importance of those imponderable intangible substances called vitamins we may well believe that every constituent of plant and animal substances, no matter in how small traces it may chance to occur, has its special significance, and we, as an association of agricultural analysts, should not rest content until we have the best possible method for its detection and determination.

As one reviews the results of agricultural chemical research, upon which reports are daily being issued from every part of the world, he

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<sup>1</sup> *Z. Nahr. Genussm.*, 1925, 49: 338.

pauses at times to wonder how much of all this work will stand the test of time. Unfortunately there is being gathered into the storehouses of chemical knowledge a vast amount of chaff, which sooner or later must be winnowed out and rejected. The elimination of all this untrustworthy material must be done by discriminating chemists, who have a thorough comprehension of analytical procedure, for a large part of these false accumulations has resulted from the employment of unreliable methods of determination. In the strenuous haste for priority in certain closely contested fields results are announced today which must be rejected and withdrawn tomorrow when a more careful repetition of the work has failed to produce confirmation. The cluttering up of the pages of our chemical journals with false and misleading information might have been entirely avoided had the authors been less precipitate and made more certain of their analytical methods.

This association does not need to be reminded that its methods of analytical control are based upon the results of most careful investigations in the field of pure science. Regulatory procedure is firmly grounded upon the conclusions of research, and our society is fortunate in having the collaboration of chemists who are investigators and those who are control officials. That research and regulatory lines of activity are necessarily repellent and antagonistic is a statement that requires considerable modification. The members of our association that are engaged in these separate fields are enabled at our meetings to give one another the benefit of mutual assistance and advice. By his suggestion of useful themes for investigation the regulatory chemist acts as a helpful guide to the research worker by pointing out fields of service that are of immediate practical importance. The contributions of the research chemist in return help to make the work of the regulatory official more reliable and more effective.

As an example of what might be done in this connection, though it may not wholly concern the work of our association, I would mention the urgent need in regulatory work of simple rapid approximate methods of analysis for moisture, fat, protein, mineral matter, etc., which can be applied as preliminary unofficial tests. By thus weeding out at the beginning all samples of genuine character, the official analyst is spared much unnecessary labor. An illustration of the saving in time which a preliminary inspectional method of analysis can accomplish is afforded by the ingenious rapid optical method of Coleman and Fellows for determining fat in grain, cattle feeds, etc. The exhibit of this method at the recent Chemical Exposition in New York attracted a vast amount of interest, which was intensified by a placard that read: "Oil Chemists save a Day. Optical Method requires 12 Minutes, A. O. A. C. Method 24 hours". By inventing other similar rapid inspectional methods of

analysis the research chemist can place himself under lasting obligations to the much overburdened control analyst.

The ambiguous wording of Article II of our constitution has unfortunately caused the chemists of some State experiment stations that are not charged with the regulatory supervision of agricultural materials to assume that they are not eligible to active membership in this association. The proper interpretation of this article and the reading of the introduction to the published *Methods of Analysis* by the honorary president of our society should convince experiment station chemists who are engaged solely in agricultural research that they are not only eligible but welcome to active membership in this organization of agricultural analysts.

We also welcome to the annual meetings of our society industrial and commercial chemists who are interested in the work of this association, the published methods of which are being used to an increasing extent in the valuation of foods and other agricultural commodities. In our fourfold relationship to the farmer, the manufacturer, the distributor, and the consumer we are sometimes less mindful of the intermediate commercial links in this chain of connection. Without being swayed by the assertions of an ultra-selfish commercialism we need always in the choice of our methods of analysis to consider carefully the industrial point of view. After many years' experience as a commercial chemist, in a general as well as in a very specialized field, I would say that an analytical method of valuation which is acceptable to both sides of a transaction is usually about as just as any that can be devised. You can rely upon the opposite instincts of buyer and seller, when large financial interests are involved, to eliminate any outstanding inaccuracy that is favorable to one side or the other and to arrive at a compromise that is equally fair to both. Where there are several neutralizing errors in a method of analysis, care must be taken in the effort for improvement lest the correction of a single defect may destroy the balance and produce a result that is less near to the truth. Not long ago a member of Congress, in a statement before the House, declared that as a result of a graduation error in the scales of saccharimeters the Government has been losing \$70,000 annually in the collection of revenue upon sugars, while the loss to producers of sugar had amounted to many millions. But he forgot to add that as a result of another opposite error of equal magnitude in raw sugar analysis, occasioned by the volume of the lead precipitate, both the Government and the producers of sugar had been gaining equally large sums of money. There exist in a number of our official methods similar instances of neutralizing errors. Both accuracy and justice require that a counterbalancing defect in a method should not be corrected unless the compensating error can be eliminated at the same time.

Commerce is naturally conservative and is hostile to innovations because of the disturbances which they create in market conditions. Many instances can be cited where the persistent adherence to obsolete commodity standards that were based upon outworn inaccurate methods of analysis has delayed analytical progress. If a method of analysis be faulty it should be corrected, if possible, immediately, and the commodity standard should then be revised to meet the new conditions. That a method of analysis should not be improved because the change would involve an alteration in commercial practices is to shackle progress and to prohibit advancement.

Analysts have always constituted the fore-guard of science. Years in advance they have forged the weapons by which progress was made against ignorance and falsehood. But how often the world has lagged in its readiness to use these weapons! Had the method of densimetric analysis, which Archimedes discovered in the third century B. C., been immediately applied the progress of science would have been greatly advanced, and the world would have been spared many of the frauds of counterfeiting and adulteration with which it was afflicted for the next 2000 years. The present book of methods of this Association of Official Agricultural Chemists contains the key to many unsuspected discoveries in science, if we only understood the application.

Liebig, who probably did more for the advancement of agriculture than any other individual in the history of mankind, also laid the foundations of modern organic analysis. Many of the pioneers in the agricultural experiment station movement of this country were also chemists, and they made important contributions in the field of analytical chemistry. But the eminent position held by Liebig, Johnson, Goessman, Hilgard, Atwater, and the others was attained because they were more than analysts. While they recognized that the chemical elements in their various combinations were the basis for the material existence of soils and crops and animals they saw also the extensive bearings of agriculture upon its social, economic, and cultural sides. The agricultural chemist of today needs more than ever the breadth of vision that these men possessed, if he is to refute the fallacious arguments of expediency which the enemies of the public welfare are so constantly advancing. The agricultural chemists of England who secured the passage of the law that prohibited the use of preservatives in milk were afterwards blamed by the opponents of this measure for an increase in price of this commodity, but they were able to prove that this increase was due not to prohibiting the use of preservatives but to rising wages and the higher costs of living.

In periods of scarcity, as during the recent war, when food and other commodities must be stretched to supply the deficiency, there is a necessary slackening in the strictness of regulatory control. The license thus

permitted is then seized upon by unscrupulous interests as an excuse for long continued infractions of the law. At such times, when the morale is lowered, when public conscience is dulled, when trade is arrogant, and when violation of the law is rampant, the control official should be fortified with every weapon at his command.

Our volume of official methods is frequently used in colleges as a textbook, and there is no discipline superior to that afforded by chemical analysis, under the direction of a good teacher, for training students in habits of observation, responsibility, and accurate thinking. It is said that Plato inscribed above the doorway of his school of philosophy in ancient Athens the words, "No one who is unfamiliar with geometry may enter". If Plato were living today he might revise his entrance requirements to, "No one who is unfamiliar with chemical analysis may enter", and he might well select agricultural analysis as the branch which offered the best opportunities for training his students in discipline, in knowledge, and in preparation for public service. In his book of Laws Plato laid down a function which is being performed by members of this association in a manner surpassing anything that this great philosopher could imagine. "The guardians of the law," he writes, "shall obtain information from experienced persons about the rogueries and adulterations of the sellers and shall write up what the seller ought and ought not to do in each case". The experienced persons, proposed by Plato, are the official agricultural chemists of today.

Chemical analysis, in the final summation, is only a means to an end. The processes described in our book of methods are simply working directions which will be employed with the highest intelligence, skill, and effectiveness only in so far as we keep in mind the ultimate purpose of our association which, whether it be for research or control, is service in the broadest and fullest meaning of the word.



## ORDER OF PUBLICATION.

The reports of the committees presented on the last day of the annual meeting will be given at the beginning of the proceedings rather than in their chronological order. This will assist the referees, associate referees, and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

### THIRD DAY.

### WEDNESDAY—MORNING SESSION.

#### REPORT OF THE REPRESENTATIVE AT THE NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH.

The fourth annual meeting of the National Conference on Pharmaceutical Research was held at Hotel Fort Des Moines, Des Moines, Iowa, on Saturday August 22, just prior to the annual meeting of the American Pharmaceutical Association.

Reports were made by the chairmen of seven of the ten standing committees of the Research Conference, those reporting and the subjects being as follows:

W. L. Scoville—Standardization of U. S. P. and N. F. Pharmaceuticals;  
H. A. B. Dunning—Manufacture of U. S. P. and N. F. Chemicals;  
C. H. LaWall—Standardization of U. S. P. and N. F. Chemicals;  
H. W. Youngken—Sources and Identification of Botanic Drugs;  
E. L. Newcomb—Standardization of Botanic Drugs;  
W. O. Emery—Chemistry of Drug Plants; and  
Ambrose Hunsberger—Business Research in Pharmacy.

The several reports were discussed at considerable length, notably the report dealing with sources of botanic drugs, reference having been made to a new and hitherto undeveloped source of *Cascara sagrada* in western Montana.

A motion directing the chairmen of all ten committees to act as a committee to report back next year as to the advisability of rearranging the ten committees with the aim of avoiding duplication of work was duly passed.

A resolution endorsing the pharmacy building campaign and urging all members of affiliated bodies to subscribe to the fund was passed by unanimous vote of the conference.

A committee consisting of Messrs. Day, Snow, and Whelpley presented

a minute of sorrow over the passing of Dean L. F. Sayre, an active supporter of the Research Conference since its inception and organization.

The census of research of 1925 was approved and the chairman directed to conduct a similar census for 1926, slight changes in the style of publication being authorized.

The question of the publication of a popular style book describing the research achievements of pharmacy was introduced by Acting-Secretary Krantz. After animated discussion, the conference endorsed the idea and directed the appointment of a committee to formulate plans and to submit same to the American Pharmaceutical Association and other national bodies likely to be interested, with a request that these organizations undertake the publication of such a book.

Under the topic of "Research Funds" attention was directed to the American Pharmaceutical Association Research Fund, the recently created Remington Research Fund of the United States Pharmaceutical Convention, and to the newly designed Ebert Medal which will replace the cash prize from the Ebert Fund awarded by the American Pharmaceutical Association each year for the best paper presented at the meeting of the association.

The nominating committee presented the following names for officers for 1925-26: Chairman, H. V. Army of New York; Vice-chairman, J. H. Webster of Detroit; secretary-treasurer, J. C. Krantz, Jr. of Baltimore, all of whom were duly elected by the conference.

The Research Conference adjourned to meet again if possible on the Saturday immediately following the 1926 meeting of the American Pharmaceutical Association.

W. O. EMERY.

Approved.

## REPORT OF THE COMMITTEE ON EDITING METHODS OF ANALYSIS.

The Committee on Editing Methods of Analysis has very little to report concerning its activities during the past year. The printing and binding of the second revision of *Official and Tentative Methods of Analysis* was completed so that delivery of the book began about June 1st of this year, and the books have been sent out at the rate of about 50 per week since that date. As would be expected in a book of the size of this revision, a few errors have been found, and the committee has decided to prepare and send to all owners of the book an errata slip indicating proper corrections. The committee requests that all members noting errors send them to the secretary of the association or to the chairman of the Committee on Editing Methods of Analysis in order that this errata

slip shall include all errors. All referees are requested to review the chapters with which they are concerned for the particular purpose of detecting the errors.

Very favorable reviews of the revised *Methods of Analysis* have appeared in the "American Chemical Journal", the English "Chemical Journal", and a number of the trade publications. The principal adverse criticisms have been directed to the multiplicity of methods for the same determination which occurs in a number of the chapters. For instance, the review in the "Journal of Chemistry and Industry" says that "one is apt to be somewhat bewildered and to ask which method of several put out is the best to adopt in particular cases". The reviewer for "Industrial and Engineering Chemistry" makes it more definite in the following statement: "As one examines the book from the standpoint of the potential user, certain points present themselves upon which comment reasonably may be made. For example, in the first chapter three methods are given for determining total nitrogen with the exclusion of nitrates—namely the Kjeldahl, the Gunning modification, and the so-called Kjeldahl-Gunning-Arnold method. All of these are indicated as official, and so presumably equal weight is given them all. The only difference between the two latter lies in the relative amounts of the sulfates used. From the practical standpoint it would seem advisable to indicate one of these methods as being most satisfactory, and, if desired to include the others, merely to indicate them by reference". In view of these comments and information received from the Committee on Recommendations of Referees, the Committee on Editing Methods of Analysis submitted to the Executive Committee of the association the following resolution:

*Whereas*, the methods of the association have been criticized for reason of a multiplicity of methods for the same determination;

*Whereas*, the methods of the association often lead to confusion in the minds of many,

*Be it therefore resolved* that it shall be the policy of the association to retain only one official method for a determination on a given product unless there be good and sufficient reason for incorporating additional methods, in which case an explanatory note shall appear in connection with the additional method.

From time to time this committee has received requests that arrangements be effected whereby new methods and additions and changes made to the official and tentative methods be made available to members of the association immediately after the close of the annual meeting. To comply with this request, the committee, in concurrence with the Board of Editors of *The Journal*, presented the following resolution to the executive committee:

The Committee on Editing Methods of Analysis respectfully recommends that arrangements be effected whereby the committee may immediately following the close of each annual meeting prepare a summary of the additions, changes, and deletions

made to the official and tentative methods of analysis, which summary shall be printed in the first issue of *The Journal* after the close of the annual meeting and that sufficient reprints of this summary shall be made to furnish each officer, referee, and associate referee with one copy and such additional copies as may be necessary for collaborative work by referees and associate referees. The Board of Editors concurs in this recommendation.

The action of the executive committee on these recommendations will be given in the report of the secretary of the association. The Committee on Editing Methods of Analysis desires again to express its appreciation to the referees, associate referees, and in fact to all members of the association who contributed so much in time and suggestions to the success of the revised book of methods. The assurance is held that it will be found a valuable contribution to the equipment of all laboratories, but it is also most earnestly requested that prompt attention be called to all errors found and all suggestions for improvement that may occur in order that these may be properly considered in future revisions.

R. E. DOOLITTLE, *Chairman*.

Approved.

## CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FORTY-FIRST ANNUAL CONVENTION, OCTOBER 26-28, 1925<sup>1</sup>.

### I. FERTILIZERS.

The zinc-iron method for the determination of nitric and ammoniacal nitrogen (p. 11) was placed under the sub-heading "Nitrogen in Nitrate Salts" (p. 12), because the method is not applicable to mixed fertilizers.

### II. SOILS.

No additions, deletions, or other changes.

### III. AGRICULTURAL LIMING MATERIALS.

No additions, deletions, or other changes.

### IV. PLANTS.

No additions, deletions, or other changes.

### V. INSECTICIDES AND FUNGICIDES.

(1) The Kissling method for the determination of nicotine in tobacco and tobacco extracts (p. 66) was dropped (final action).

(2) The xylene distillation method for the determination of water in soaps was adopted as a tentative method. The method is as follows:

<sup>1</sup> As compiled by the Committee on Editing Methods of Analysis, R. E. Doolittle, Chairman. Unless otherwise stated, all references in this report are to *Methods of Analysis*, A. O. A. C., 1925.

Weigh about 20 grams of the sample into a 300-500 cc. flask; add 50 cc. of xylene; and, to prevent foaming, add about 10 grams of lump rosin. (Do not use powdered rosin, as it usually contains an appreciable quantity of moisture.) Distil into a Dean and Stark type distilling tube receiver<sup>1</sup>. Continue the distillation until no more water collects in the receiver. Allow the contents of the tube to cool to room temperature, read the volume of water under the xylene in the tube, and from this volume calculate the percentage of water in the sample.

(3) The following methods for the examination of mineral oil-soap emulsions were adopted as tentative methods:

(a) *Water (Xylene Distillation Method).—Tentative.*

Weigh about 25 grams of the sample into a 300-500 cc. flask; add 50 cc. of xylene and, if necessary to prevent foaming, a small piece of rosin; and proceed as directed in the determination of water in soaps, beginning with "Distil into a Dean and Stark distilling tube receiver".

(b) *Total Oil<sup>2</sup>.—Tentative.*

Weigh about 10 grams of the sample into a Babcock cream bottle. Dilute with about 10 cc. of hot water and add 5-10 cc. of dilute sulfuric acid (1 + 1). Set the bottle in a hot water bath for about 5 minutes to hasten the separation of the oil, add sufficient saturated sodium chloride solution to bring the oil layer within the graduations on the neck of the bottle, whirl at a rate of 1200 revolutions per minute for 5 minutes, and allow to cool. Read the volume of the oily layer, determine its density, and from these values calculate its weight and percentage. From this percentage value, deduct the percentage of fatty acids (and phenols if present) determined separately, to obtain the percentage of oil in the sample.

(c) *Soap.—Tentative.*

Weigh 20 grams of the sample into a separatory funnel, add 60 cc. of petroleum ether, and extract the mixture once with 20 cc. and four times with 10 cc. of 50 per cent alcohol. Break the emulsion if necessary with 1 or 2 cc. of a strong solution of sodium hydroxide, allowing the solution to run down the side of the separatory funnel, which is then gently twirled and allowed to stand for a few minutes. Draw off the alcoholic layers and wash them successively through petroleum ether contained in two other separatory funnels. Combine the alcoholic extracts in a beaker and evaporate on a steam bath to remove the alcohol. Dissolve the residue in about 100 cc. of water made alkaline with sodium hydroxide. Transfer to a separatory funnel, acidify with hydrochloric or sulfuric acid, extract three times with ether, and wash the ether extracts twice with water. Combine the ether extracts, evaporate in a weighed beaker on a steam bath, and weigh as fatty acids. From the weight of fatty acids calculate the percentage of soap in the sample as sodium or potassium oleate.

(d) *Unsulphonated Residue.—Tentative.*

REAGENT.

38 *N* sulfuric acid.—Prepare as directed on p. 408, 83.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 486.

<sup>2</sup> *U. S. Dept. Agr. Bur. Chem. Bull.* 105, p. 165.

## DETERMINATION.

Measure 5 cc. of the recovered oil into a Babcock cream bottle. To this add slowly 20 cc. of the 38 *N* sulfuric acid. Mix the contents gradually, being careful that the temperature does not rise above 60°C. When the mixture no longer warms on shaking, agitate thoroughly; place on a water bath; and heat to 60°–65°C. for 10 minutes, keeping the contents of the flask thoroughly mixed by shaking vigorously five or six times. Fill the flask with strong sulfuric acid until the oil rises into the graduated neck. Centrifugalize 4–5 minutes at about 1200 revolutions per minute. Read the volume of unsulfonated residue from the graduations on the neck of the bottle and from this value calculate the percentage by volume of the unsulfonated oil.

(e) *Ash.—Tentative.*

Evaporate 10 grams of the sample, or more, if necessary, in a platinum dish; ignite; and leach the charred mass with water. Ignite the residue, add the leachings, evaporate to dryness, ignite, and weigh. From this weight calculate the percentage of ash. Test the ash for copper, calcium, calcium fluoride, etc.

## VI. TANNING MATERIALS.

No additions, deletions, or other changes.

## VII. LEATHERS.

No additions, deletions, or other changes.

## VIII. WATERS, BRINE, AND SALT.

(1) The method for the determination of hydrogen sulfide in waters (p. 93) was dropped (final action) and the following method was adopted as official (final action).

## HYDROGEN SULFIDE—OFFICIAL.

## REAGENTS.

(a) *Hydrochloric acid.*

(b) *Starch indicator.*—Prepared as directed on p. 48, 3 (e).

(c) *0.02 N iodine solution.*—Dissolve 10 grams of potassium iodide (free from iodic acid) in a liter flask, using as little water as possible. Add 2.54 grams of resublimed iodine and dissolve by shaking. Dilute to the mark with water. Standardize against a thiosulfate solution that has been recently standardized against a potassium dichromate solution.

(d) *0.01 N iodine solution.*—Mix equal volumes of reagent (c) and boiled water. Standardize against a thiosulfate solution as directed under (c).

## DETERMINATION.

Transfer a quantity of the sample to a graduated vessel by means of a siphon and add a few drops of phenolphthalein indicator. If alkaline, add hydrochloric acid (a) until the pink color of the indicator disappears. Add starch indicator and, with careful stirring, titrate with iodine solution, (c) or (d), until a permanent blue color appears. Correct for the quantity of iodine solution needed to give an equally blue color. From the corrected quantity of iodine solution used, calculate the approximate quantity of hydrogen sulfide present. For accurate determinations siphon 100–500 cc. of the sample, according to the quantity of hydrogen sulfide present, into a graduated vessel, keeping the outlet of the siphon below the liquid. Add immediately a sufficient quantity of hydrochloric acid (a), calculated from the approximate determination, to make neutral to phenolphthalein indicator. Mix carefully with a bent glass rod and without

delay add about 0.5 cc. less iodine reagent (c) or (d) than is needed to combine with the hydrogen sulfide present. Add 5 cc. of starch indicator (b), and finish the titration with iodine solution drop by drop with stirring until a blue color remains permanently. Correct for the quantity of iodine solution needed to give an equally blue color when the same quantity of starch solution is added to an approximately equal volume of boiled water. If possible, make several determinations and take an average. Standardize reagents (c) and (d) frequently.

## IX. FEEDING STUFFS.

(1) The following distillation method for the determination of water in cattle feeds was adopted as a tentative method:

### MOISTURE.

*By Distillation<sup>1</sup>.—Tentative.*

#### APPARATUS.

This apparatus consists of a 250 cc. distilling flask of Pyrex or other resistant glass connected by means of a "distilling tube receiver"<sup>2</sup> to a 20 inch sealed-in straight-tube Liebig condenser with delivery tube not over  $\frac{5}{8}$  inch in the manner shown in Fig. 1. The "distilling tube receiver" is of the dimensions shown and is made by attaching a proper side tube to the calibrated section of a 5 cc. Mohr pipet and sealing the outlet. The tube is calibrated in tenths of a cubic centimeter by distilling known quantities of water into the graduated column, and the column of water may be read to hundredths with reasonable accuracy. Clean the tube and condenser with chromic-sulfuric acid mixture, rinse thoroughly with water, then with alcohol, and dry in an oven to prevent an undue quantity of water adhering to the inner surfaces during the determination.

#### DETERMINATION.

If the sample is likely to bump, add enough dry sand to cover the bottom of the flask. Add sufficient toluene to cover the sample completely, usually about 75 cc. Weigh and introduce into the toluene sufficient sample to give 2-5 cc. of water and connect the apparatus as shown in Fig. 1. Fill the receiving tube with toluene by pouring through the top of the condenser. Bring to a boil and distil slowly, about 2 drops per second, until most of the water has passed over; then increase the rate of distillation to about 4 drops per second. When the water is apparently all over, wash down the condenser by pouring toluene in at the top, continuing the distillation a short time to ascertain whether any more water will distil over; if it does, repeat the washing down process. If any water remains in the condenser, remove it by brushing down with a tube brush attached to a copper wire and

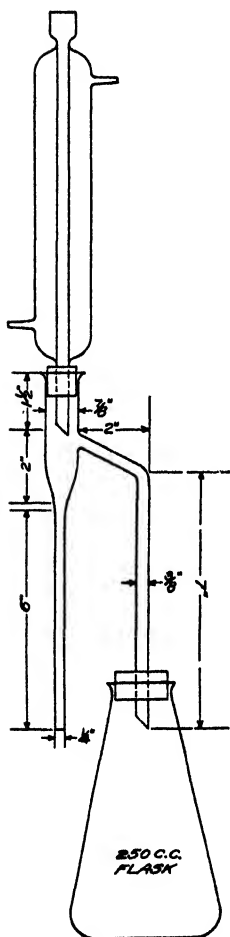


FIG. 1.  
APPARATUS FOR THE  
DETERMINATION OF  
MOISTURE.

<sup>1</sup> *This Journal*, 1925, 8: 295.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1920, 12: 486.

saturated with toluene, washing down the condenser at the same time. The entire process is usually completed within an hour. Allow the receiving tube to come to room temperature. If any drops adhere to the sides of the tube, force them down by means of a rubber band wrapped around a copper wire. Read the volume of water and calculate to percentage.

(2) The tentative method for the determination of starch in the presence of interfering polysaccharides (such as linseed meal) was made official (final action). The method is as follows:

#### STARCH.

(In the presence of interfering polysaccharides<sup>1</sup>.)

#### REAGENT.

*Malt Extract.*—Use clean, new barley malt of known efficacy and grind only as needed. Grind well, but not so fine that filtration will be greatly retarded. Prepare an infusion of the freshly ground malt, just before it is to be used. For every 80 cc. of the malt extract required digest 5 grams of the ground malt with 100 cc. of water, at room temperature, for 2 hours, or for 20 minutes if the mixture can be stirred by an electric mixer. Filter to obtain a clear extract. (It may be necessary to return the first portions of the filtrate to the filter.) Mix the infusion well.

#### DETERMINATION.

Weigh 2–6 grams (charges of 4 grams for linseed meal, or 3 grams for dried apple pomace, have been found to be satisfactory) of the well-mixed sample, prepared to pass freely through a sieve not less than 40 mesh to the inch, using the smaller charges in the case of materials containing much gel-forming substance. (The weight of starch in the charge must not exceed 1.5 gram.) Transfer to a dry 12.5–15 cm. close-textured rapid filtering paper in a glass funnel and extract with 5 successive portions of ether, taking for each portion more than enough to cover the charge and using a cover glass to retard evaporation. After completing the ether extraction, allow the last traces of ether to evaporate and then extract the charge with 300 cc. of dilute alcohol. The concentration of the alcohol may be varied somewhat to suit the material under examination. For linseed meal use 35 per cent alcohol (by volume) and for dried apple pomace use 25 per cent alcohol. Follow this with several filterfuls of 95 per cent alcohol and finish the leaching operations with a second ether extraction. Conduct also a control determination, preferably in duplicate, using a filter paper extracted with alcohol and the same quantity of water and malt extract as in the determination. (It is convenient to let the charge stand overnight at this point to allow the ether and alcohol to evaporate, as alcohol must be eliminated before starting the digestion with malt.)

Transfer as much of the dry material as possible from the filter paper into a glass mortar and pulverize all lumps. Transfer both filter paper and sample to a 500 cc. volumetric flask, add 20–30 cc. of water, and thoroughly wet the material by vigorous shaking.

Should more cold water be needed to make the material more fluid, calculate the quantity of hot water to be added accordingly, so the total volume allowing for 40 cc. of malt solution will not exceed 200 cc. Let stand a few minutes and then add 100 cc. of actively boiling water. The sample now must be thoroughly gelatinized at boiling temperature in a water bath.

Cool to 50°C. or lower, add 20 cc. of the malt extract to controls as well as to charges, and place the flasks in a temperature-controlled water bath. Keeping the mash thor-

<sup>1</sup> *J. Agr. Research*, 1923, 23: 995.



oughly mixed, gradually raise the temperature to 70°C. in 20-30 minutes. Maintain at 70°C. for 30 minutes, stirring the mixture from time to time, then increase the temperature to 80°C., and keep it at that temperature for 10 minutes. Finally heat to the boiling point. Keep the mixtures well stirred. Cool the contents of the flasks and the water bath to 55°C. Add 20 cc. of the malt extract, mix well, and hold at 55°C. for 1 hour, stirring about once every 10 minutes. At the termination of the digestion rapidly increase the temperature to above 80°C.

Measure out 316 cc. of 95 per cent alcohol. Add a portion, a little at a time, to the contents of the flask, with thorough shaking between additions. After cooling to room temperature adjust the volume with water so that the quantity of liquid is 500 cc., making allowance for the volume occupied by the charge by adding 3 cc. of water for every 4 grams of charge present, after bringing the contents to the 500 cc. mark. Mix thoroughly, breaking up any ropy coagulum as much as possible by pouring back and forth from one large beaker to another. Filter through dry paper. Test the solid residue for starch, either microscopically or by the iodine color test, after elimination of alcohol and gelatinization with water. (If more than the merest trace of starch is found, reject the *entire determination*.) Evaporate exactly 200 cc. of the filtrate on a steam bath to a volume of 15-20 cc., or until practically all alcohol has been expelled. Do not allow the evaporation to proceed to dryness.

Transfer the aqueous residue of starch conversion products to a 200 cc. volumetric flask with hot water, using a rubber-tipped rod to recover any dextrine that may be present. Allow to cool somewhat, and complete the volume to 200 cc. Transfer the contents to a suitable digestion flask, add 20 cc. of dilute hydrochloric acid (sp. gr. 1.125) made by diluting 68 cc. of strong acid (sp. gr. 1.19, or 37 per cent HCl) to 100 cc., and connect the flask with a reflux condenser. Heat in a boiling water bath for 2.5 hours. Cool and, for samples of linseed meal or other material yielding solutions which at this stage need further purification, add not more than 1 cc. of a 10 per cent solution of phosphotungstic acid in 1 per cent hydrochloric acid. Mix, and allow to stand at least 15 minutes. Increase the volume with water to 250 cc. in a volumetric flask, mix well, and filter through dry paper. Partially neutralize 200 cc. of the filtrate while stirring by adding 10 cc. of a strong solution of caustic soda (44 grams of sodium hydroxide per 100 cc. of the cooled solution) and nearly complete the neutralization with a little powdered anhydrous sodium carbonate. Transfer to a 250 cc. flask with water, cool to room temperature, make up to the mark, and thoroughly mix. Filter, if necessary, and determine the dextrose in a 50 cc. aliquot of the filtrate, gravimetrically, as directed on page 196, 56 or 58. Correct the weight of dextrose obtained by the weight of dextrose found for the same aliquot of the malt control, and multiply the corrected weight of dextrose by 0.90 to obtain the weight of starch.

Aliquots,

$$\text{Charge} \times \frac{200}{300} \times \frac{200}{250} \times \frac{50}{250}, \text{ or}$$

$$\text{Charge} \times 0.064.$$

(3) The following method for the detection of added salt in feeding stuffs was adopted as an official method (first action).

#### SALT.<sup>1</sup>

Transfer 2 cc. of a 5 per cent solution of silver nitrate to a small test tube of 1 cm. internal diameter. Carefully add to this liquid an equal volume of the feed, which previously has been ground to pass a millimeter sieve, so that most of the sample floats or

<sup>1</sup> *This Journal*, 1924, 7: 344.

remains above the liquid. Gradually incline the tube so that the liquid is absorbed. White patches of silver chloride appear wherever the minutest crystal of salt comes in contact with the solution. These patches may easily be observed with a lens or even with the naked eye.

## X. PRESERVATIVES AND ARTIFICIAL SWEETENERS.

No additions, deletions, or other changes.

## XI. COLORING MATTERS IN FOODS.

No additions, deletions, or other changes.

## XII. METALS IN FOODS.

No additions, deletions, or other changes.

## XIII. SUGARS AND SUGAR PRODUCTS.

(1) The basic divisor in the formula for calculating sucrose in the absence of raffinose by polarization before and after inversion with invertase (p. 186) was changed from 142.0 to 142.1 (first action).

(2) The method for the determination of sucrose in the absence of raffinose by polarization before and after inversion with invertase (p. 183), including inversion at room temperature and at 55°–60°C. and formula corrected as above was adopted as official (final action).

(3) The following method for the determination of sucrose and raffinose in the presence of each other by use of two enzyme preparations was adopted as an official method (first action).

### DETERMINATION OF SUCROSE AND RAFFINOSE.

*By Polarization before and after Treatment with two Enzyme Preparations.—Tentative.*

#### REAGENTS.

(a) *Invertase solution (top yeast extract).*—Prepare as directed on p. 183, **21**. This solution should be free from the enzyme melibiase. Its invertase activity should be at least as great as when used for the determination of sucrose in the absence of raffinose [p. 184, **21** (4)].

(b) *Invertase-melibiase solution (bottom yeast extract).*—Prepare as directed on p. 183, **21**, using bottom fermenting yeast (brewers yeast) instead of bakers yeast. The invertase activity should be at least as great as in (a).

Test the melibiase activity of the solution as follows: Add 2 cc. of the solution to be tested to 20 cc. of a weakly acid melibiose solution polarizing + 20.0°V. and allow to stand 30 minutes at about 20°C. Then add sufficient sodium carbonate to render the solution slightly alkaline to litmus paper. A preparation suitable for the overnight hydrolysis of solutions containing not more than 0.2 gram of raffinose in 100 cc. should have hydrolyzed 35 per cent of the melibiose present under the conditions mentioned; a preparation suitable for the overnight hydrolysis of solutions containing not more than 0.65 gram of raffinose in 100 cc. should have produced 50 per cent hydrolysis of melibiose; and a preparation suitable for the overnight hydrolysis of solutions containing 0.65 to 1.3 gram raffinose in 100 cc. should have hydrolyzed at least 70 per cent of the melibiose present under the above conditions.

## DETERMINATION.

In the analysis of sugar beet products, weigh the quantity of material specified in Table 1, transfer to a 300 cc. volumetric flask, add the quantity of basic lead acetate solution indicated in Table 1, and dilute to volume at 20°C. Mix thoroughly and filter through fluted paper in a closely covered funnel, rejecting the first 25 cc. of filtrate. When sufficient filtrate has collected, remove the lead from the solution by adding ammonium dihydrogen phosphate in as small excess as possible (see Table 1). This condition is readily determined after a little practice by the appearance of the lead phosphate precipitate, which usually flocculates and settles rapidly in the presence of a slight excess of the salt. Mix well and filter, again rejecting at least the first 25 cc. of the filtrate. Polarize direct in a 200 mm. tube at 20°C. unless the solution contains an appreciable quantity of invert sugar, in which case pipet a 50 cc. portion of the lead-free filtrate into a 100 cc. flask, dilute with water to the mark, mix well, and polarize at 20°C., preferably in a 400 mm. tube. This reading, calculated to the normal weight of 26 grams in 100 cc., and 200 mm. tube length is the direct reading (*P*) of the formula given below for polarization before inversion.

Transfer two 50 cc. portions of the lead-free filtrate to 100 cc. flasks. To one add 5 cc. of invertase solution (top yeast extract) and to the other 5 cc. of invertase-melibiase solution (bottom yeast extract), let stand overnight at atmospheric temperature (preferably not below 20°C.), dilute to volume, mix well, and polarize at 20°C., preferably in

TABLE 1.

*Quantities of sample and reagents required for clarification and deleading of beet sugar-house products.*

MATERIAL	QUANTITY PER 100 CC.	BASIC LEAD ACETATE (55° BRIX)	AMMONIUM DIHYDROGEN PHOSPHATE
	grams	cc.	gram
Cosettes* . . . . .	13	3	0.2
Pulp . . . . .	100 cc. <sup>b</sup>	2-4	0.2
Lime cake or sewer <sup>c</sup> . . . . .	26.5	1.5	... <sup>d</sup>
Thin juice . . . . .	52	2	0.2-0.3
Thick juice . . . . .	26	4	0.3-0.4
White massecuite . . . . .	13 or 26	3 or 6	0.3-0.7
High wash sirup . . . . .	13 or 26	3 or 6	0.3-0.7
High green sirup . . . . .	13 or 26	5 or 10	0.3-0.7
Raw or remelt massecuite . . . . .	13	6	0.3-0.4
Raw or remelt sugar . . . . .	26	3-4	0.3-0.4
Sugar melter . . . . .	26	2-3	0.3-0.4
Low wash sirup . . . . .	13	8-10	0.4-0.5
Low green sirup or molasses . . . . .	13	10	0.4-0.5
Saccharate cakes and milk (carbonated) . . . . .	26	4-6	0.3-0.4
Steffen waste and wash waters <sup>e</sup> . . . . .	78 or 50 cc.	2-3	0.2

\* Usual method of extraction, 26 grams in 201.2 cc.

<sup>b</sup> Dilute to 110 cc.

<sup>c</sup> Neutralize with acetic acid before adding basic lead acetate.

<sup>d</sup> Lime in solution will be partly precipitated by the phosphate, and it is necessary to add sufficient phosphate to complete the precipitation of both the lead and lime salts; hence no definite quantity can be specified.

a 400 mm. jacketed tube. If a rapid hydrolysis is desired, add 10 cc. of each of the enzyme solutions to the 50 cc. portions of delead filtrate in 100 cc. flasks and place in a water bath at 50°-55°C. for 40 minutes. Then add sodium carbonate until the solution is slightly alkaline to litmus paper, dilute to volume at 20°C., mix well, and polarize at 20°C., preferably in a 400 mm. tube. Correct the invert readings for the optical

activity of the enzyme solutions, and calculate the polarization to that of a normal weight solution of 26 grams in 100 cc.; also calculate the reading to a 200 mm. tube length, if necessary.

Calculate the percentages of anhydrous raffinose and sucrose from the following formulas:

$$R = 1.354 (A - B);$$

$$S = \frac{(P - 2.202A + 1.202B) 100}{132.12 - 0.00718 [132.12 - (P - 2.202A + 1.202B)]},$$

Where  $R$  = percentage of raffinose;

$S$  = percentage of sucrose;

$A$  = corrected polarization after top yeast hydrolysis; and

$B$  = corrected polarization after bottom yeast hydrolysis.

The quantities  $A$  and  $B$  are treated algebraically.

(4) The Lane-Eynon method for the volumetric determination of reducing sugars<sup>1</sup> was adopted as a tentative method. The method is as follows:

*Lane-Eynon General Volumetric Method.—Tentative.*

REAGENTS.

The reagents and solutions are described on p. 189, 29.

STANDARDIZATION AND METHOD OF TITRATION.

Pipet accurately 10 or 25 cc. of mixed Soxhlet's reagent or pipet 5 or 12.5 cc. of each of Soxhlet's solutions (a) and (b) into a flask of 300–400 cc. capacity. The quantity of copper taken will differ slightly between the two methods of pipetting, and the method employed must be carried out consistently during standardization and determination. Prepare a standard solution of the pure sugar of such concentration that more than 15 cc. and less than 50 cc. will be required to reduce all the copper. The titer may be calculated by  $\frac{\text{factor}}{\text{mg. sugar in 1 cc.}} = \text{titer}$ . Add almost the whole of the sugar solution

required to effect reduction of all the copper, so that not more than 0.5–1 cc. is required later to complete the titration. Heat the cold mixture to boiling on a wire gauze and maintain in moderate ebullition for two minutes, lowering the flame sufficiently to avoid lumping. Without removing the flame add 2–5 drops of 1 per cent aqueous methylene blue solution and complete the titration within a total boiling time of about 3 minutes by small additions of sugar solution to decolorization of the indicator.

Multiply the titer by the number of milligrams in 1 cc. of the standard solution to obtain the factor. Compare with the tabulated factor to determine the correction, if any, to be applied to the table. Small deviations from the tabulated factors may arise from variations in individual procedure or composition of reagents. If only approximate results (within 1 per cent) are required the standardization may be omitted, provided the specifications of the analysis are rigidly observed.

DETERMINATION.

If the approximate concentration of the sugar in the sample is unknown, proceed by the incremental method of titration. Add to 10 or 25 cc. of Soxhlet's solution 15 cc. of the sugar solution and heat to boiling over a wire gauze. Boil about 15 seconds and

<sup>1</sup> *J. Soc. Chem. Ind.*, 1923, 42: 32T.

judge from the appearance of the mixture if a further 10 cc. portion of sugar may be added. When it is judged that the copper is approaching exhaustion, continue the boiling for 1-2 minutes from the commencement of ebullition; then add 2-5 drops of methylene blue and complete the titration by small additions of sugar. The results of this titration will, in general, be in error by not more than 1-2 per cent.

For analysis of higher precision repeat the titration, adding almost the whole of the sugar solution required to reduce all the copper and proceed as described under "Standardization and Method of Titration". In the table find the factor corresponding to the titer and apply the correction previously determined. Estimate the sugar by

$$\frac{\text{Factor} \times 100}{\text{titer}} = \text{mg. of sugar in 100 cc.}$$

TABLE 1.

*Factors for 10 cc. Sozihel's solution to be used in connection with the Lane-Eynon general volumetric method.*

TITER	NO SUCROSE INVERT SUGAR	1 GRAM SUCROSE PER 100 CC. INVERT SUGAR	5 GRAMS SUCROSE PER 100 CC. INVERT SUGAR	10 GRAMS SUCROSE PER 100 CC. INVERT SUGAR	25 GRAMS SUCROSE PER 100 CC. INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	50.5	49.9	47.6	46.1	43.4	49.1	52.2	77.2	81.3	64.9	68.3
16	50.6	50.0	47.6	46.1	43.4	49.2	52.3	77.1	81.2	64.8	68.2
17	50.7	50.1	47.6	46.1	43.4	49.3	52.3	77.0	81.1	64.8	68.2
18	50.8	50.1	47.6	46.1	43.3	49.3	52.4	77.0	81.0	64.7	68.1
19	50.8	50.2	47.6	46.1	43.3	49.4	52.5	76.9	80.9	64.7	68.1
20	50.9	50.2	47.6	46.1	43.2	49.5	52.5	76.8	80.8	64.6	68.0
21	51.0	50.2	47.6	46.1	43.2	49.5	52.6	76.7	80.7	64.6	68.0
22	51.0	50.3	47.6	46.1	43.1	49.6	52.7	76.6	80.6	64.6	68.0
23	51.1	50.3	47.6	46.1	43.0	49.7	52.7	76.5	80.5	64.5	67.9
24	51.2	50.3	47.6	46.1	42.9	49.8	52.8	76.4	80.4	64.5	67.9
25	51.2	50.4	47.6	46.0	42.8	49.8	52.8	76.4	80.4	64.5	67.9
26	51.3	50.4	47.6	46.0	42.8	49.9	52.9	76.3	80.3	64.5	67.9
27	51.4	50.4	47.6	46.0	42.7	49.9	52.9	76.2	80.2	64.4	67.8
28	51.4	50.5	47.7	46.0	42.7	50.0	53.0	76.1	80.1	64.4	67.8
29	51.5	50.5	47.7	46.0	42.6	50.0	53.1	76.0	80.0	64.4	67.8
30	51.5	50.5	47.7	46.0	42.5	50.1	53.2	76.0	80.0	64.4	67.8
31	51.6	50.6	47.7	45.9	42.5	50.2	53.2	75.9	79.9	64.4	67.8
32	51.6	50.6	47.7	45.9	42.4	50.2	53.3	75.9	79.9	64.4	67.8
33	51.7	50.6	47.7	45.9	42.3	50.3	53.3	75.8	79.8	64.4	67.8
34	51.7	50.6	47.7	45.8	42.2	50.3	53.4	75.8	79.8	64.4	67.9
35	51.8	50.7	47.7	45.8	42.2	50.4	53.4	75.7	79.7	64.5	67.9
36	51.8	50.7	47.7	45.8	42.1	50.4	53.5	75.6	79.6	64.5	67.9
37	51.9	50.7	47.7	45.7	42.0	50.5	53.5	75.6	79.6	64.5	67.9
38	51.9	50.7	47.7	45.7	42.0	50.5	53.6	75.5	79.5	64.5	67.9
39	52.0	50.8	47.7	45.7	41.9	50.6	53.6	75.5	79.5	64.5	67.9
40	52.0	50.8	47.7	45.6	41.8	50.6	53.6	75.4	79.4	64.5	67.9
41	52.1	50.8	47.7	45.6	41.8	50.7	53.7	75.4	79.4	64.6	68.0
42	52.1	50.8	47.7	45.6	41.7	50.7	53.7	75.3	79.3	64.6	68.0
43	52.2	50.8	47.7	45.5	41.6	50.8	53.8	75.3	79.3	64.6	68.0
44	52.2	50.9	47.7	45.5	41.5	50.8	53.8	75.2	79.2	64.6	68.0
45	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.2	79.2	64.7	68.1
46	52.3	50.9	47.7	45.4	41.4	50.9	53.9	75.1	79.1	64.7	68.1
47	52.4	50.9	47.7	45.3	41.3	51.0	53.9	75.1	79.1	64.8	68.2
48	52.4	50.9	47.7	45.3	41.2	51.0	54.0	75.1	79.1	64.8	68.2
49	52.5	51.0	47.7	45.2	41.1	51.0	54.0	75.0	79.0	64.8	68.2
50	52.5	51.0	47.7	45.2	41.0	51.1	54.0	75.0	79.0	64.9	68.3

TABLE 2.

*Factors for 25 cc. Soxhlet's solution to be used in connection with the Lane-Eynon general volumetric method.*

TITER	NO SUCROSE INVERT SUGAR	1 GRAM SUCROSE PER 100 CC. INVERT SUGAR	DEXTROSE	LEVULOSE	ANHYDROUS MALTOSE $C_{12}H_{22}O_{11}$	HYDRATED MALTOSE $C_{12}H_{22}O_{11} \cdot H_2O$	ANHYDROUS LACTOSE $C_{12}H_{22}O_{11}$	HYDRATED LACTOSE $C_{12}H_{22}O_{11} \cdot H_2O$
15	123.6	122.6	120.2	127.4	197.8	208.2	163.9	172.5
16	123.6	122.7	120.2	127.4	197.4	207.8	163.5	172.1
17	123.6	122.7	120.2	127.5	197.0	207.4	163.1	171.7
18	123.7	122.7	120.2	127.5	196.7	207.1	162.8	171.4
19	123.7	122.8	120.3	127.6	196.5	206.8	162.5	171.1
20	123.8	122.8	120.3	127.6	196.2	206.5	162.3	170.9
21	123.8	122.8	120.3	127.7	195.8	206.1	162.0	170.6
22	123.9	122.9	120.4	127.7	195.5	205.8	161.8	170.4
23	123.9	122.9	120.4	127.8	195.1	205.4	161.6	170.2
24	124.0	122.9	120.5	127.8	194.8	205.1	161.5	170.0
25	124.0	123.0	120.5	127.9	194.5	204.8	161.4	169.9
26	124.1	123.0	120.6	127.9	194.2	204.4	161.2	169.7
27	124.1	123.0	120.6	128.0	193.9	204.1	161.0	169.5
28	124.2	123.1	120.7	128.0	193.6	203.8	160.8	169.3
29	124.2	123.1	120.7	128.1	193.3	203.5	160.7	169.2
30	124.3	123.1	120.8	128.1	193.0	203.2	160.6	169.0
31	124.3	123.2	120.8	128.1	192.8	202.9	160.5	168.9
32	124.4	123.2	120.8	128.2	192.5	202.6	160.4	168.8
33	124.4	123.2	120.9	128.2	192.2	202.3	160.2	168.6
34	124.5	123.3	120.9	128.3	191.9	202.0	160.1	168.5
35	124.5	123.3	121.0	128.3	191.7	201.8	160.0	168.4
36	124.6	123.3	121.0	128.4	191.4	201.5	159.8	168.2
37	124.6	123.4	121.1	128.4	191.2	201.2	159.7	168.1
38	124.7	123.4	121.2	128.5	191.0	201.0	159.6	168.0
39	124.7	123.4	121.2	128.5	190.8	200.8	159.5	167.9
40	124.8	123.4	121.2	128.6	190.5	200.5	159.4	167.8
41	124.8	123.5	121.3	128.6	190.3	200.3	159.3	167.7
42	124.9	123.5	121.4	128.6	190.1	200.1	159.2	167.6
43	124.9	123.5	121.4	128.7	189.8	199.8	159.2	167.6
44	125.0	123.6	121.5	128.7	189.6	199.6	159.1	167.5
45	125.0	123.6	121.5	128.8	189.4	199.4	159.0	167.4
46	125.1	123.6	121.6	128.8	189.2	199.2	159.0	167.4
47	125.1	123.7	121.6	128.9	189.0	199.0	158.9	167.3
48	125.2	123.7	121.7	128.9	188.9	198.9	158.8	167.2
49	125.2	123.7	121.7	129.0	188.8	198.7	158.8	167.2
50	125.3	123.8	121.8	129.0	188.7	198.6	158.7	167.1

(5) The Soxhlet volumetric method for the determination of reducing sugars (p. 190, **32, 33**) was dropped.

(6) The method for the determination of copper by electrolytic deposition from sulfuric acid solution (p. 192, **40**) was dropped.

(7) The method for the determination of copper by electrolytic deposition from nitric acid solution (p. 193, **42**) was dropped.

(8) The method for the electrolytic deposition of copper from sulfuric acid solution was rewritten in order to supply the details formerly incorporated in Paragraph **40** (p. 190). This method will then read as follows:

*Electrolytic Deposition from Sulfuric and Nitric Acid Solution<sup>1</sup>.—Official.*

Decant the hot solution through an asbestos mat in a Gooch crucible and wash the beaker and precipitate thoroughly with hot water. Transfer the asbestos film from the crucible to the beaker by means of a glass rod and rinse the crucible with about 30 cc. of a boiling mixture of dilute sulfuric and nitric acids containing 65 cc. of concentrated sulfuric and 50 cc. of strong nitric acid per liter. Heat, and agitate until solution is complete and the oxides of nitrogen have been volatilized. Filter, and transfer to a weighed platinum dish. Dilute to about 100 cc. and deposit the copper by electrolysis at 20°–30°C., using about 2.5 volts and a current density of about 0.5 ampere. Cover the dish with a split watch glass to avoid loss by spattering. Electrolysis requires about 14 hours, but it may be allowed to continue overnight. Test 1 cc. with strong H<sub>2</sub>S water for complete deposition. Wash thoroughly with water; then break the current, wash with alcohol and ether successively, dry at about 50°C., and weigh.

If preferred, the electrolysis can be conducted in a beaker, the copper being deposited upon a weighed platinum foil or platinum gauze.

**XIV. FRUITS AND FRUIT PRODUCTS.**

The tentative method for the determination of added water in white grape juice (p. 218)<sup>2</sup> was modified to read as follows:

Transfer about 50 cc. of the filtered juice to a 2 ounce tincture bottle containing ten glass rods, 15 mm. long and 5 mm. in diameter, and approximately one gram of finely powdered cream of tartar. Cork the bottle tightly and place it neck downward in a pint Mason jar. Fill the Mason jar with water of 25°C. and hold at this temperature for 30 minutes, shaking occasionally. (By placing the jar in a bucket of water, the temperature of 25°C. can be readily controlled.) At the end of this time seal the Mason jar, wrap it in three sheets of heavy wrapping paper, making three separate wrappings, place in a mechanical shaker, and shake for 1 hour. After the shaking has been accomplished, ascertain the temperature "*t*" of the water in the Mason jar. Filter the juice immediately and titrate 10 cc. with 0.1 *N* sodium hydroxide, using phenolphthalein indicator. Titrate in the same manner 10 cc. of the original juice. The two titrations should be made side by side in order to obtain the same shade of pink with the greatest possible accuracy. Calculate the percentage of added sugar solution (water) from the following formula:

$$W = \frac{0.0188(b - a) - 0.095 - 0.025 \left( \frac{2}{t^\circ - 25} \right)}{0.006}, \text{ in which}$$

*W* = percentage of volume of added water (20 per cent sugar solution);

*b* = acidity of treated juice, cc. 0.1 *N* sodium hydroxide per 100 cc.;

*a* = acidity of original juice, cc. 0.1 *N* sodium hydroxide per 100 cc.; and

*t*° = temperature after shaking.

Pure factory juices examined by this method show a small quantity of added water varying from one to three per cent.

**XV. CANNED VEGETABLES.**

No additions, deletions, or other changes.

<sup>1</sup> Price and Meade. Analysis of Brass, 1917.

<sup>2</sup> This Journal, 1925, 8: 724.

## XVI. CEREAL FOODS.

The following methods for the examination of cereal foods were adopted:

## FLOUR.

## DIRECTIONS FOR SAMPLING.—TENTATIVE.

Sample a number of sacks equivalent to the square root of the number in the lot, but not less than ten, i. e., ten from 100 or less, fifteen from 225, twenty from 400 sacks, etc.

Select the sacks to be sampled according to their exposure in the ratio of four from the most exposed, three from the next less exposed, two from the next, and one from the least exposed portion of the lot.

From each sack to be sampled, draw a core from one corner of the top diagonally to the center of the sack by means of a cylindrical, pointed, polished steel trier, one-half inch in diameter, with a slit of at least one-third the circumference. Draw a second core from the other top corner to one-half the distance to the center of the sack.

Deliver the two cores at once to a clean, dry, air-tight container which has stood open for a few minutes near the lot of flour to be sampled and seal immediately. Use a separate container for each sack sampled. One of the following containers may be used: (1) One pint Mason jar provided with a rubber gasket; (2) a rubber pouch which can be tied or sealed to exclude moisture or air; (3) a tin can or box with a moisture and air-tight friction top.

Before opening the sample for analysis, alternately invert and roll each container twenty-five times, or more if necessary, to secure a homogeneous mixture. Avoid excessive temperatures and humidities when opening for analysis. Keep the sample tightly sealed at all other times.

For such supplemental determinations as net weight, uniformity, and baking tests samples may be increased in number, increased in quantity, or combined to suit the requirements of the analyst if the principles laid down in these directions are followed.

## TOTAL SOLIDS.

## MOISTURE (INDIRECT METHOD).

1. *Vacuum Method*<sup>1</sup>.—*Tentative.*

## APPARATUS.

(a) *Metal dish*.—Diameter about 55 mm., height about 15 mm., provided with an inverted slip-in cover fitting tightly on inside.

(b) *Air-tight desiccator*.—Should contain reigned quick lime or calcium carbide.

(c) *Vacuum oven*.—Should be connected with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm. or less of mercury and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. A concentrated sulfuric acid gas drying bottle is connected with the oven for admitting dry air for releasing the vacuum.

(d) *Mercury manometer*.—Used to indicate the pressure of the partial vacuum.

## DETERMINATION.

Weigh accurately about 2 grams of the well mixed sample in a covered dish that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after attaining room temperature. Loosen the cover (do not remove) and heat at 98°–100°C. to constant weight (approximately 5 hours) in a partial vacuum having a pressure

<sup>1</sup> *This Journal*, 1925, 8: 665.



equivalent to 25 mm. or less of mercury. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temperature is attained. Report the flour residue as total solids and the loss in weight as moisture (indirect method).

## II. Routine Air-Oven Method<sup>1</sup>.—Tentative.

(This method gives results closely approximating those by the vacuum method.)

### APPARATUS.

- (a) *Metal dish and desiccator*.—As described under I—the *Vacuum Method*.
- (b) *Oven*.—Should be capable of being maintained at approximately 130°C. ( $\pm 3^\circ$ ) and provided with an opening for ventilation.
- (c) *Thermometer*.—To be placed with its bulb near the samples.

### DETERMINATION.

Weigh accurately approximately 2 grams of the well-mixed sample in a covered dish that has been dried previously at approximately 130°C. ( $\pm 3^\circ$ ), cooled in the desiccator, and weighed soon after attaining room temperature. Uncover the sample and dry the dish, cover, and contents in the oven at approximately 130°C. ( $\pm 3^\circ$ ) for 1 hour. Cover the dish while still in the oven, transfer to the desiccator, and weigh soon after room temperature is attained. Report the flour residue as total solids and the loss in weight as moisture (indirect method).

### WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.—TENTATIVE.

#### REAGENTS.

- (a) *40 per cent alcohol*.—Mix 50 volumes of water and 35 volumes of 95 per cent alcohol.
- (b) *Asbestos*.—Ignite and rub through an 8-mesh sieve.

### DETERMINATION.

Place 20 grams of the flour in a 200 cc. nursing bottle, add 100 cc. of water from a pipet, shake the bottle violently a few times to prevent lumping of the sample, and add exactly 100 cc. more of water. Shake the stoppered bottle in a mechanical shaker (if not available, shake by hand) for 30 minutes. (The temperature of the water should not exceed 30°C.) Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. (The filtrate should be practically clear.) Pipet 100 cc. of the filtrate into a 250 cc. beaker-flask or Erlenmeyer flask. Add 1.2 grams of sodium chloride and dissolve. Add 0.1 gram of asbestos, shake, and with constant agitation add 70 cc. of 95 per cent alcohol. Allow to stand overnight. Filter the mixture through a thin pad of asbestos in a Gooch crucible, using light suction. Wash the flask and precipitate twice with the 40 per cent alcohol. Transfer the filter mat with the precipitate to a Kjeldahl flask and determine the nitrogen as directed on p. 8, 22, collecting the ammonia in 10 cc. of 0.1 *N* acid. Make blank determinations on the reagents, using as nearly as possible the same quantity of asbestos.

### LIPIDS AND LIPOID PHOSPHORIC ACID ( $P_2O_5$ ).

#### LIPIDS.—TENTATIVE.

Add 15 cc. of alcohol, 70 per cent by volume, to 5 grams of the flour, in a 200 cc. nursing bottle. Give the bottle a gentle rotary motion so as to moisten all the particles

<sup>1</sup> *This Journal*, 1925, 8: 665.

with the alcohol, stopper, and set in a water bath kept at 75°–80°C. Heat for 15 minutes with frequent mixing by the same rotary motion. Add 27 cc. of 95 per cent alcohol, stopper the bottle, and shake vigorously for 2 minutes. Cool, add 45 cc. of ether, and shake well for 5 minutes. (The sample should now be in a fine state of division.) Centrifugalize just sufficiently to throw the solid particles out of suspension but not so as to pack the sample too firmly. Decant the liquid into a 250 cc. beaker containing some bits of broken porcelain or glass, and rinse off the bottle neck with ether. Re-extract the sample with three successive 20 cc. portions of ether, shake 1 or 2 minutes each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts just to dryness on the steam bath. Drive off any remaining moisture on the sides of the beaker by placing in a boiling water oven for 5 minutes. Dissolve the dry extract in approximately 15 cc. of chloroform and filter the solution into a previously dried and weighed platinum dish through a pledget of cotton packed in the stem of a funnel. Free with a glass rod any solid extract adhering to the beaker and transfer through the filter into the first washings by means of chloroform from a wash bottle all extract from the beaker bottom and sides. Finally wash the funnel and stem tip. The filtrate should be perfectly clear. Evaporate the chloroform on a steam bath and dry the dish and contents in a boiling water oven until no more weight is lost (75–90 minutes). Weigh. Report the extract as lipoids.

#### LIPOID PHOSPHORIC ACID ( $P_2O_5$ ).—TENTATIVE.

Dissolve the lipoids in 5–10 cc. of chloroform, add 5–10 cc. of 4 per cent alcoholic potassium hydroxide solution, evaporate to dryness on a steam bath, and char well in a furnace at a faint red heat. Cover the dish with a cover glass, add sufficient dilute nitric acid (1 + 9) to make the solution slightly acid, warm on a steam bath, and filter. Wash the residue and filter well with hot water. Determine phosphoric acid in the filtrate as directed on p. 3, 7 or 10. Report as lipid phosphoric acid ( $P_2O_5$ ).

#### FAT (ACID HYDROLYSIS METHOD).—TENTATIVE.

Place 2 grams of the flour in a 50 cc. beaker, add 2 cc. of 95 per cent alcohol, and stir so as to moisten all particles. (The moistening of the sample with alcohol prevents lumping on addition of the acid.) Add 10 cc. of dilute hydrochloric acid (25 + 11), mix well, set the beaker in a water bath held at 70°–80°C., and stir at frequent intervals for 30–40 minutes. Add 10 cc. of 95 per cent alcohol and cool. Transfer the mixture to a Röhrig or Mojonier fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc. of ethyl ether in three portions and shake the mixture well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.) and mix well. Let stand until the upper liquid is practically clear. Draw off as much as possible of the ether-fat solution through a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether into a weighed 125 cc. beaker-flask containing some porcelain chips or broken glass. Before weighing the beaker-flask dry it in an oven at the temperature of boiling water and then allow it to stand in the air to constant weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc. of each ether. Shake well on the addition of each ether. Draw off the clear ether solutions through the filter into the same flask as before and wash the tip of the spigot, the funnel, and end of the funnel stem with a few cc. of a mixture of the two ethers in equal volumes free from suspended water. Evaporate the ethers slowly on a steam bath, then dry the fat in a boiling water oven until it ceases to lose weight (approximately 75 minutes). Remove the flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. Correct this weight by a blank determination on the reagents used.

**BAKED CEREAL PRODUCTS.**

(1) The sub-heading "BREAD" was inserted immediately under the heading "BAKED CEREAL PRODUCTS" (p. 230).

(2) The tentative method for the determination of moisture in baked cereal products (p. 230) was dropped.

(3) The following methods for the examination of bread were adopted:

**PREPARATION OF SAMPLE.—TENTATIVE.**

(To be used when total solids of original entire loaf is not desired.)

Cut the loaf of bread into slices 2-3 mm. thick. Spread the slices on paper and allow them to dry in a warm room until sufficiently crisp and brittle to grind well in a mill. Grind the entire sample just to pass a 20-mesh sieve, mix well, and keep in an air-tight container.

**TOTAL SOLIDS IN AN ENTIRE LOAF OF BREAD.—TENTATIVE.**

Accurately weigh the loaf of bread immediately upon receipt (A). Use scales sensitive to at least 0.2 gram. (When determining whether bread is in conformity with the Department of Agriculture standards do not weigh the loaf sooner than one hour after removal from the oven.) Should accurate weighing be impossible at this time, seal the sample in an air-tight container and accurately weigh as soon thereafter as is practicable (A). Preserve the sample in such a manner that no loss of bread solids can occur, whereby the loss would be calculated as moisture. Cut the bread into slices 2-3 mm. thick. Spread the slices on paper; allow them to dry in a warm room (approximately 15-20 hours); and when apparently dry, break into fragments. If the bread is not entirely crisp and brittle, allow it to dry longer—until it is in equilibrium with the moisture of the air—in order that no moisture changes may occur during grinding. Quantitatively transfer the air-dried bread to the scale pan and accurately weigh (B). Grind the sample just to pass a 20-mesh sieve, mix well, and keep in an air-tight container. Determine the percentage of total solids (C) of the ground sample as directed under the vacuum method for total solids in flour. Calculate total solids of the bread from the formula—

$$TS = \frac{\frac{B \times C}{100}}{A} \times 100, \text{ or } \frac{B \times C}{A}, \text{ in which}$$

A = weight of loaf at time of receipt,

B = weight of the air-dried sliced loaf of bread; and

C = percentage of total solids in the prepared ground sample.

**TOTAL SOLIDS OF AIR-DRIED GROUND SAMPLE.—TENTATIVE.**

Use 2 grams of sample prepared as directed under "Preparation of Sample" and proceed as directed under the vacuum method for flour.

**ASH.—TENTATIVE.**

Use 3-5 grams of sample prepared as directed under "Preparation of Sample" or "Total Solids in an Entire Loaf of Bread" and proceed as directed under the official method for ash in flour.

**PROTEIN.—OFFICIAL.**

Determine nitrogen as directed on p. 7, 19, or p. 8, 22 or 24, using 2 grams of air-dried ground sample prepared as directed under "Preparation of Sample" or "Total Solids in an Entire Loaf of Bread". Multiply the percentage of nitrogen by the factor 5.7 to obtain the percentage of protein.

**ALIMENTARY PASTES.**

The following additions and changes were made to the methods for the examination of alimentary pastes under cereal products:

**COLLECTION AND PREPARATION OF SAMPLE.—TENTATIVE.**

The tentative method (p. 231) was dropped, and in its place the following tentative method was substituted:

Pick out sufficient strips or pieces from the lot to be analyzed to assure a representative sample. Break these into small fragments with the hands or in a mill and mix well. Weigh 400–500 grams (A) accurately to 2 decigrams, grind in a mill until all the material just passes through a 20-mesh sieve, and reweigh (B). (The operations of grinding and sifting should be done quantitatively to the same degree of accuracy as the weighings.) Keep the ground sample in a sealed container to prevent moisture changes.

If the total solids of the original unground material is of no concern, then the weighings (A) and (B) may be omitted and less care exercised to avoid material losses during the grinding and sifting.

**TOTAL SOLIDS AND MOISTURE (INDIRECT METHOD).—TENTATIVE.**

The present tentative method for moisture (p. 231) was dropped, and the following tentative method was substituted:

Determine the total solids (C) and moisture (indirect method) in the sample, prepared as directed under "Collection and Preparation of Sample", by the vacuum method for total solids and moisture (indirect method) in flour.

If the total solids of the original unground material is desired then calculate as follows:

Let A = weight of sample before grinding;

B = weight of ground sample; and

C = percentage of total solids in the prepared ground sample.

Then the percentage of total solids in the original unground material (T. S.) =

$$\frac{\frac{B \times C}{100} \times 100}{A}, \text{ or } \frac{B \times C}{A}, \text{ and the}$$

percentage of moisture (indirect method) in the original unground material =  
100 - (T. S.).

**ASH.—TENTATIVE.**

The tentative method for the determination of ash in alimentary pastes (p. 232) was made official (first action).

**CHLORIDES IN ASH AS SODIUM CHLORIDE.—TENTATIVE.**

The tentative method for the determination of chlorides in the ash of alimentary pastes (p. 232) was made official (first action).

**ORGANIC AND AMMONIACAL NITROGEN.—TENTATIVE.**

The tentative method for the determination of organic and ammoniacal nitrogen (p. 232) was made official (first action).

**PROTEIN.—TENTATIVE.**

The following method for the determination of protein in alimentary pastes was adopted as official (first action).

Multiply the percentage of organic and ammoniacal nitrogen by the factor 5.7 to obtain the percentage of protein.

**EXTRACTION AND IDENTIFICATION OF ADDED COLOR.—TENTATIVE.**

The tentative method for the extraction and identification of added color in alimentary pastes (p. 233) was made official (first action).

**XVII. MEAT AND MEAT PRODUCTS.**

No additions, deletions, or other changes.

**XVIII. GELATIN.**

No additions, deletions, or other changes.

**XIX. DAIRY PRODUCTS.**

(1) The cryoscopic method for the detection of added water in milk (p. 265) was adopted as a tentative method for the detection of added water in cream.

(2) The following method for the determination of moisture in cheese was adopted as an official method (first action).

**MOISTURE IN CHEESE.****APPARATUS.**

*Metal dish.*—Diameter about 55 mm., height about 15 mm., provided with a slip-in inverted cover fitting tightly on the inside.

**DETERMINATION.**

Weigh 2–3 grams of the prepared sample into the previously weighed metal dish, cover tightly, and again weigh. In the case of soft cheese of high moisture content, weigh 1–2 grams and partially dry on a steam bath. Dry the cheese in the loosely covered dish, placed in direct contact with the metal shelf of the oven, to constant weight (approximately 4 hours) under a pressure not to exceed 100 mm. (4 inches) of mercury at the temperature of boiling water. During the drying admit into the oven a slow current of air (about 2 bubbles per second) dried by passing through concentrated sulfuric acid. Discontinue the action of the vacuum pump and carefully re-admit air into the oven. Press the cover tightly into the dish, remove the dish from the oven, cool, and weigh. Express the loss in weight as moisture.

**XX. FATS AND OILS.**

(1) The official method for the determination of unsaponifiable residue in fats and oils (p. 295) was dropped (first action).

(2) The tentative Kerr-Sorber method<sup>1</sup> for the determination of unsaponifiable matter in fats and oils was dropped.

<sup>1</sup> *This Journal*, 1925, 8: 272.

(3) The F. A. C. method<sup>1</sup> for the determination of unsaponifiable matter was adopted as official (first action). The method is as follows:

**UNSAPONIFIABLE RESIDUE.—TENTATIVE.**

**REAGENT.**

*Petroleum ether.*—Redistilled below 75°C. Make a blank determination by evaporating 350 cc. of the reagent with about 0.25 gram of stearine or other hard fat (previously brought to constant weight by heating) and drying as in the actual determination. The blank must not exceed a few milligrams.

**APPARATUS.**

*Extraction cylinder.*—The cylinder shall be glass-stoppered, graduated at 40 cc., 80 cc., and 130 cc., and of the following dimensions; diameter about 1½ inches, height about 12 inches.

**DETERMINATION.**

Weigh 5 grams ( $\pm 0.020$  gram) of the prepared sample into a 200 cc. Erlenmeyer flask, add 30 cc. of redistilled 95 per cent (approximately) by volume ethyl alcohol and 5 cc. of 50 per cent aqueous potassium hydroxide, and boil the mixture for one hour under a reflux condenser. Transfer to the extraction cylinder and wash to the 40 cc. mark with redistilled 95 per cent ethyl alcohol. Complete the transfer, first with warm, then with cold water, until the total volume is 80 cc. Cool the cylinder and contents to room temperature and add 50 cc. of the petroleum ether. Shake as vigorously as possible for one minute and allow to settle until both layers are clear, when the volume of the upper layer should be about 40 cc. Draw off the petroleum ether layer as closely as possible by means of a slender glass siphon into a separatory funnel of 500 cc. capacity. Repeat the extraction at least six more times, using 50 cc. of petroleum ether for each extraction. Wash the combined extracts into a separatory funnel three times with 25 cc. portions of 10 per cent alcohol by volume, shaking vigorously each time. Transfer the petroleum ether extract to an Erlenmeyer weighed flask; distil; or, if desired, evaporate the petroleum ether on a steam bath in a current of air. Heat the flask with residue until a constant weight is obtained in an oven at a uniform temperature not less than 100°C. nor more than 110°C. (A vacuum oven may be used at a corresponding temperature, which depends upon the pressure employed in it. It is important to displace with air any residue vapors of petroleum ether remaining in the flask after heating, before it is weighed.) Deduct any blank from the weight before calculating unsaponifiable matter. Test the final residue for solubility in 50 cc. petroleum ether at room temperature. Filter, and wash free from the insoluble residue, if any. Evaporate and dry in the same manner as before.

**XXI. BAKING POWDERS AND BAKING CHEMICALS.**

(1) The tentative electrolytic method for the determination of lead in baking powder and baking chemicals (p. 310) was made official (first action).

(2) The tentative method for the determination of fluorides (p. 312) was modified as follows and continued as a tentative method. The last three sentences of the method were deleted and the following substituted therefor:

<sup>1</sup> *J. Ind. Eng. Chem.*, 1919, 11: 1161; *This Journal*, 1924, 8: 85; 1925, 8: 484.

After titration make the neutral solution to a definite volume and divide into two equal parts. Determine sulfates in one portion as directed on p. 45, 17, and chlorides in the other as directed on p. 87, 21. Calculate the results to the whole sample in terms of 0.1 *N* alkali and correct accordingly.

Weight of  $\text{BaSO}_4 \times 82.75 = \text{cc. of } 0.1 \text{ } N \text{ alkali.}$

Cc. of  $\text{AgNO}_3 \times 0.282 = \text{cc. of } 0.1 \text{ } N \text{ alkali.}$

Multiply the corrected result by the factor 1.1 to obtain the fluorine content.

## XXII. SPICES AND OTHER CONDIMENTS.

No additions, deletions, or other changes.

## XXIII. VINEGARS.

(1) The method for the determination of total reducing substances before inversion (p. 326) was made official (final action).

(2) The method for the determination of total reducing substances after inversion (p. 326) was made official (final action).

(3) The tentative method for the determination of lead precipitate (p. 329) was dropped.

## XXIV. COFFEE.

No additions, deletions, or other changes.

## XXV. TEAS.

No additions, deletions, or other changes.

## XXVI. CACAO PRODUCTS.

The Lepper-Waterman method<sup>1</sup> for the determination of fat in cacao products was adopted as an official method (first action). This method is as follows:

Prepare in a Knorr extraction tube a tightly packed mat of asbestos purified as for the determination of crude fiber [p. 117, 15 (C)] and carefully freed from coarse pieces. Wash the filter with alcohol, ether, and a little petroleum ether. (*All petroleum ether used in this determination must be redistilled below 60°C.*) Weigh 2–3 grams of the sample, prepared as directed under 1, into the tube. Insert the tube into a rubber stopper in a filtering bell-jar connected to the suction through a two-way stopcock, taking care that no rubber particles adhere to the tip of the stem. Place a weighed 150 cc. Erlenmeyer flask at such a height that the tube stem passes through the neck into the flask. (The stem of the tube should be lengthened if necessary.) Fill the tube to about two-thirds of its capacity with the redistilled petroleum ether, and by means of a rod having a flattened end stir the sample thoroughly, taking care to crush all lumps. Let stand 1 minute and drain by suction. Regulate the suction so that the collected solvent in the flask will not boil violently. Add the solvent from a wash-bottle, at the same time turning the tube between thumb and finger so that the sides of the tube are washed down by each addition. Repeat the extractions, with stirring, until the fat is removed. (Ten extractions will usually be sufficient.) Remove the tube

<sup>1</sup> *This Journal*, 1925, 8: 706.

with stopper from the bell, wash the traces of fat from the end of the stem with petroleum ether, evaporate the solvent, and dry to constant weight at 100°C.

The fat-free sample may be used for the crude fiber determination.

## XXVII. FLAVORING EXTRACTS.

The Wichmann method<sup>1</sup> for the determination of lead number in vanilla extract and its substitutes, with an alternative procedure for determining the lead as lead chromate, was adopted as an official method (final action.) The method is as follows:

### LEAD NUMBER (WICHMANN).—OFFICIAL.

#### REAGENTS.

(a) *Lead acetate solution*.—Dissolve 80 grams of neutral lead acetate in water that has been recently boiled, dilute to 1 liter, and filter if the solution is not clear.

(b) *Potassium dichromate solution, approximately 0.01 N*.—Dissolve 5 grams of pure crystallized potassium dichromate ( $K_2Cr_2O_7$ ) in water and dilute to 1 liter.

#### DETERMINATION.

Place 175 cc. of boiled water in a round bottom flask of 1 liter capacity. Add by means of pipets 25 cc. of the lead acetate solution and 50 cc. of the sample. Place the flask in a hole in an asbestos board that is large enough to prevent the heating of the upper portion of the flask. (The hole in the board should be of such size that when the flask contains 50 cc. of liquid, the level of the liquid will be even with the top of the board, or slightly above it.) Connect the flask to a condenser, and with a moderate flame distil 200 cc. into a volumetric flask. Calculate the approximate alcohol content of the extract from the specific gravity of the distillate. (For accurate results, redistil over alkali.) Transfer the residual solution to a 100 cc. volumetric flask by means of carbon dioxide-free water and a bent glass rod provided with a rubber tip. When cool, dilute to 100 cc. with carbon dioxide-free water, mix, and filter through a dry filter (Solution A).

Pipet 10 cc. of Solution A into a 250 cc. beaker and add 25 cc. of water, 2 cc. of dilute sulfuric acid (1 + 1), and 100 cc. of 95 per cent alcohol. Stir, and allow to settle overnight. Filter on a Gooch crucible, wash with 95 per cent alcohol, dry, ignite at low redness, cool in a desiccator, and weigh. For the blank determination, proceed as before, but use 5 drops of glacial acetic acid in place of the sample and distil 150 cc. instead of 200 cc. The difference between the two weights of lead sulfate multiplied by 13.6646 gives the lead number of the extract. Report as "Lead number—Wichmann".

Or, pipet 10 cc. of the clear filtrate from the lead precipitate (Solution A) into a 400 cc. beaker and add 2 cc. of glacial acetic acid, 25 cc. of water, and 25 cc. of the potassium dichromate solution. Heat the beaker and contents immediately with a moderate flame and continue heating until the precipitate changes in color from yellow to orange. Then filter the solution through a weighed Gooch crucible provided with an asbestos mat and wash thoroughly with hot water and then with a few cc. each of alcohol and ether. Dry at 100°C., cool in a desiccator, and weigh. Determine the lead in the blank in the same manner. The difference in weights of lead chromate multiplied by 12.8217 is the lead number.

<sup>1</sup> J. Ind. Eng. Chem., 1921, 13: 414; This Journal, 1925, 8: 689.



**VANILLA RESINS.**

The following method for the determination of resins in vanilla extracts was adopted as a tentative method and inserted immediately following the heading (p. 350) "Vanilla Resins".

*Quantitative Test.—Tentative.*

Pipet 50 cc. of the extract into a small beaker; add 50 cc. of water; evaporate to 50 cc. on a steam bath; add 50 cc. of water; and again evaporate to 50 cc. Cool. If the mixture has an acid reaction, add 2 cc. of dilute hydrochloric acid (1 + 1). If the mixture is not acid to litmus, add dilute hydrochloric acid (1 + 1), drop by drop, until distinctly acid to litmus paper, then 1 cc. in excess. Cover and let stand overnight. Filter; wash 6 or 7 times with approximately 0.05 *N* hydrochloric acid [9 cc. hydrochloric acid (1 + 1) per liter of water]. Dissolve the resin in warm 95 per cent alcohol by pouring through the filter. Evaporate the alcohol in a tared 50 cc. beaker and dry to constant weight at 100°C. Report results to two decimal places only. Reserve the resin for qualitative tests.

The "Qualitative Test.—Tentative" (p. 350) was changed as follows:

Paragraph 1 of Section 11 reading "Place 50 cc. of the extract \* \* \* filtrate for further tests" was deleted. The first sentence of Paragraph 2 of Section 11, reading "Place a portion of the filter with the attached resins" was changed to read, "Place a portion of the dried residue". The modified method is to be inserted immediately following the sub-heading "Qualitative Tests.—Tentative".

**XXVIII. WINES.**

No additions, deletions, or other changes.

**XXIX. DISTILLED LIQUORS.**

No additions, deletions, or other changes.

**XXX. BEERS.**

No additions, deletions, or other changes.

**XXXI. DRUGS.****ACETYSALICYLIC ACID.**

(1) The tentative qualitative test for free salicylic acid in acetylsalicylic acid (p. 387) was made official (first action).

(2) The tentative quantitative method (p. 387) for the determination of free salicylic acid in acetylsalicylic acid was made official (final action).

(3) The tentative bromine method for the determination of total salicylates in acetylsalicylic acid (p. 388) was made official (final action).

(4) The tentative double titration method for the determination of acetylsalicylic acid (p. 388) was modified to read as follows:

## PREPARATION OF SOLUTION.

(a) *Dry extraction method (applicable in all cases).*—Treat a quantity of sample containing not less than 0.3 gram of acetylsalicylic acid, accurately weighed, with small portions of chloroform, filter into a beaker, and wash with chloroform until completely extracted. Evaporate the bulk of the chloroform on a steam bath, finishing with the aid of an electric fan without heat.

(b) *Wet extraction method (applicable in the absence of acids and alkaline or alkali earth carbonates).*—Transfer the accurately weighed sample to a small separatory funnel containing about 20 cc. of water. Shake out repeatedly with chloroform, using successively 30, 25, 20, 15, 10, and 5 cc. portions and test for completeness of extraction by evaporating on a watch glass a portion of the final extraction. Filter the combined chloroform portions through cotton and wash the funnel and cotton with chloroform. Evaporate the bulk of the chloroform on a steam bath, finishing with the aid of an electric fan without heat.

(c) *For samples of acetylsalicylic acid and uncoated tablets containing no excipient.*—Dissolve the sample directly in 10 cc. of alcohol.

## DETERMINATION.

(a) *Single titration method.*—To the dry chloroform extract in a 250 cc. Erlenmeyer flask, add from a pipet 25 cc. of approximately 0.5 *N* alcoholic potassium hydroxide solution. At the same time measure into another 250 cc. flask a blank using the same pipet and draining for the same length of time. Place a funnel in the neck of each flask and boil the contents gently on a steam bath for about 15 minutes. Cool, and titrate the sample and the blank with 0.5 *N* acid, using phenolphthalein indicator. Each cc. of 0.5 *N* potassium hydroxide solution consumed in the saponification equals 0.0450 gram of acetylsalicylic acid.

(b) *Double titration method.*—Dissolve the dry chloroform extract in 10 cc. of neutral alcohol and titrate immediately with 0.1 *N* alkali solution, using phenolphthalein indicator. Make this titration rapidly and use the first persistent pink color as the end point, since any slight excess of alkali has a tendency to hydrolyze the ester quickly. Add a volume of the 0.1 *N* alkali equal to that used in the first titration and then add 5 cc. more. Heat on a steam bath for 15 minutes. Titrate back with 0.1 *N* acid. If the product is pure, the total quantity of alkali consumed will be twice that of the first titration. Each cc. of 0.1 *N* alkali consumed in the two titrations is equivalent to 9 mg. of acetylsalicylic acid.

(5) The following method<sup>1</sup> for the determination of combined acetic acid and salicylic acid was adopted as official (first action).

## COMBINED ACETIC ACID.—TENTATIVE.

Weigh accurately 2 grams of the powdered material and transfer to a separatory funnel, using about 25 cc. of water. Extract completely with chloroform, testing the last extraction by evaporating a small quantity of the chloroform to dryness. (Usually

<sup>1</sup> *This Journal*, 1925, 8: 506.

six extractions of 30, 25, 20, 10, 10, and 5 cc. portions of chloroform are sufficient.) Collect the chloroform fractions in a beaker and filter through a pledget of cotton into a weighed beaker, which has been counterpoised previously with a beaker of the same dimensions, similarly dried and exposed to the air. Wash the original beaker, funnel, and cotton with chloroform and add these washings to the chloroform solution in the weighed beaker. Evaporate the chloroform on a steam bath, dry the residue at 80°C. for 15 minutes, and weigh, using the counterpoised beaker similarly treated. From the weight calculate the chloroform extract.

Treat the chloroform extract, or if no excipients are present 2 grams of the powdered material, in a 150 cc. beaker with 30 cc. of normal sodium hydroxide and evaporate on a steam bath nearly to dryness. Transfer to a separatory funnel, using for this operation 10 cc. of water, 20 cc. of dilute sulfuric acid (6 + 94), and finally two 5 cc. portions of water. Extract with successive portions of chloroform, using the first fraction of 50 cc. to rinse the beaker in which the saponification was carried on. Continue the extractions with chloroform until all salicylic acid is removed. (This generally requires about six extractions.) During these extractions keep the stopper in the funnel to guard against loss of acetic acid by evaporation. Collect the chloroform fractions in a second separatory funnel and wash with 25 cc. of water and this wash water once with 5 cc. of chloroform. Discard the chloroform extractions and return the wash water to the acid water in the first funnel. Transfer the acid water containing acetic and sulfuric acids to a 200 cc. volumetric flask, wash the separatory funnels thoroughly with water, add to the flask, dilute to volume, and mix thoroughly. Pipet two 50 cc. portions, using the same pipet and draining it the same length of time. Place one portion in a receptacle suitable for titration and the other in a large platinum dish. Titrate the first portion at once with 0.5 *N* alkali, using phenolphthalein indicator. Evaporate the portion in the platinum dish on a steam bath to dryness, take up in 10 cc. of water, and again evaporate, repeating this process twice more. (During evaporation guard against contact with ammonia vapors.) Take up the residue in water and titrate with 0.5 *N* alkali, using phenolphthalein indicator. Subtract the second titration reading from the first and calculate the percentage of acetic acid on a 0.5 gram sample.

1 cc. of 0.5 *N* alkali is equivalent to 0.030015 gram of acetic acid.

(6) The following method for the determination of acetylsalicylic acid in mixtures containing acetphenetidin and caffeine<sup>1</sup> was adopted as official (final action).

#### ACETYSALICYLIC ACID IN MIXTURES CONTAINING ACETPHENETIDIN AND CAFFEINE.—OFFICIAL.

Ascertain the average weight of a number of tablets and reduce to a fine powder.

Weigh accurately 0.2 gram of the powder, transfer to a separatory funnel with about 25 cc. of water, and extract carefully with repeated portions of chloroform. Test the final extraction by evaporating a small portion on a steam bath to dryness. (No residue should remain if the extraction is complete. About six extractions are generally required, and these can be made with 30, 25, 20, 10, 10, and 5 cc. portions of chloroform.) Collect the chloroform fractions in a separatory funnel and draw off into a 200 cc. Erlenmeyer flask, placing a pledget of cotton in the stem of the funnel to filter the chloroform. Wash the funnel twice with 5 cc. portions of chloroform, passing this through the cotton and leaving any water that may have separated in the funnel. Add the chloroform washings to the flask and evaporate the chloroform on a steam bath to a volume of about 2 cc. Add 10 cc. of sulfuric acid (1 + 9), connect with a reflux con-

<sup>1</sup> *This Journal*, 1925, 8: 506.

denser, and digest for 30 minutes, partially immersing the flask in a boiling water bath. Cool, and transfer to a separatory funnel, rinsing the condenser with chloroform and using a minimum quantity of water to effect the transfer, so that the final volume does not greatly exceed 20 cc. Extract the caffeine and salicylic acid with six portions of chloroform, using 30, 25, 20, 15, 10, and 10 cc. for the extractions. Collect these fractions in a separatory funnel, add 20 cc. of water and 1 gram of sodium carbonate, and shake thoroughly. Drain off the chloroform into another separator and wash twice more with 15 and 10 cc. of water. Reject the chloroform and combine the sodium carbonate solution and wash waters in a 200 cc. Erlenmeyer flask. Heat on a steam bath to expel traces of chloroform, dilute to 100 cc. with water, then add slowly 25-40 cc. of strong iodine solution (about 0.2 *N*), sufficient to insure excess during digestion, and digest one hour on the steam bath. Remove the free iodine with a few drops of sodium thiosulfate solution. Decant the clear solution through a weighed Gooch, retaining most of the precipitate in the flask. To the latter add 50 cc. of boiling water; digest 10 minutes on the steam bath; filter; and wash gradually all the precipitate into the Gooch, using altogether about 200 cc. of hot water to complete the operation. Dry to constant weight in an air bath at 100°C. and weigh the precipitate of tetraiodophenylenequinone ( $C_6H_2I_4O$ )<sub>2</sub>. Weight of precipitate times 0.4016 gives the total salicylic acid present. If free salicylic acid is present, this should be deducted from the total, and the difference times 1.304 is the weight of acetylsalicylic acid.

#### ARSENICALS.

The following method for the determination of arsenic in sodium cacodylate was adopted as a tentative method.

Transfer 0.2 gram of the sample accurately weighed to a kjeldahl flask. Conduct a blank using the same quantities of reagents. Add 10 grams of potassium sulfate, 0.3 gram of starch, and 20 cc. of concentrated sulfuric acid. Digest over a low flame until frothing has ceased. Continue the digestion 4 hours or until the mixture is colorless. Cool, dilute with water, and transfer to a 500 cc. Erlenmeyer flask. Add sodium hydroxide solution (1 + 1) slowly until alkaline to litmus paper and acidify with sulfuric acid. Place the flask in water until thoroughly cooled, add 5 grams of sodium bicarbonate, and titrate with 0.1 *N* iodine solution.

1 cc. of 0.1 *N* iodine solution is equivalent to 0.00575 gram of arsenic or 0.008 gram of anhydrous sodium cacodylate.

#### BARBITAL AND PHENOBARBITAL.

The tentative method for the estimation of barbital and phenobarbital<sup>1</sup> was made official (first action). The method is as follows:

#### REAGENTS.

(a) *Alkaline salt solution*.—Dissolve 20 grams of sodium hydroxide in water, dilute to 1 liter, add sodium chloride to saturation, and filter.

(b) *Solvent*.—Mix 20 cc. of alcohol, 10 cc. of ether, and 70 cc. of chloroform.

#### PREPARATION OF SAMPLE.

If in tablets, ascertain the average weight and powder a representative number.

<sup>1</sup> *This Journal*, 1925, 8: 48, 510.

## DETERMINATION.

Transfer 0.3 gram of the powdered sample to a separatory funnel. Dissolve in 10 cc. of the alkaline salt solution. If tablet lubricants are present, wash with 15 cc. of absolute ether and decant from top of separatory funnel into a small beaker. Add 2 cc. of strong hydrochloric acid to the alkaline solution, then 5 cc. of water to prevent supersaturation of salt. Extract five times, using 30, 20, 20, 10, and 10 cc. portions of the solvent. Combine the solvent in a second separatory funnel and wash with 2 cc. of water acidified with a drop of hydrochloric acid. Filter the solvent through a pledget of cotton into a small weighed beaker. Evaporate on a steam bath with the aid of an electric fan. Heat 10 minutes at 90°–100°C., cool in a desiccator, and weigh. Test for complete extraction with 10 cc. of solvent and evaporate in a separate beaker.

## CAMPHOR.

(1) Tentative Method I for the determination of monobromated camphor in tablets (p. 393) was made official (first action).

(2) The following method for the determination of camphor was adopted as a tentative method.

Weigh accurately into a 400 cc. round-bottomed pyrex flask, a sufficient quantity of the powdered substance to contain approximately 2 grams of camphor. Add 10 cc. of benzol and 10 cc. of water and connect the flask with an apparatus for steam distillation. Use an 8–12 inch bulb condenser, well cooled, the outlet of which reaches to the bottom of a 200 cc. flask. Distil with steam, collecting the benzol and about 100 cc. of aqueous distillate. Disconnect the condenser and wash it slowly with 5 cc. of alcohol from a pipet in such a manner as entirely to wet the inside of the condenser. Wash the condenser in the same manner with 10 cc. of benzol. Add both washings to the contents of the receiver. Saturate the distillate with sodium chloride, add sufficient dilute sulfuric acid (1 + 9) to insure acidity, transfer to a separatory funnel, shake, and separate the two layers. Rinse the original receiver with 10 cc. of benzol and with the rinsings re-extract the aqueous solution. Separate the aqueous layer and extract it once more with 10 cc. of benzol. Wash the combined benzol portions with 10 cc. of saturated salt solution rendered distinctly alkaline with sodium carbonate. Separate the layers and extract the aqueous layer with 10 cc. of benzol. Discard the aqueous solutions, transfer the benzol portions to a 50 cc. volumetric flask, and make up to the mark. Shake the solution and filter it into a 200 mm. polariscope tube, using a water jacketed tube, if necessary, in order to maintain a constant temperature of 20°C. Make 10 readings, using a bichromate filter, and take the average reading for calculating the camphor. Calculate the quantity of camphor ( $Q$ ) contained in the 50 cc. of benzol and, therefore, in the sample taken, from the average reading in circular degrees ( $a$ ) by the following formula:

$$Q = 0.6171 a - 0.0022 a^2.$$

The value of  $Q$  does not vary directly with the length of the tube. In the event that a longer or shorter tube is used than directed, the value of ( $a$ ) must be corrected to a 200 mm. tube, and the calculation then made in accord with the above formula.<sup>1</sup>

## CHAULMOOGRA OIL.

The following methods for the examination of chaulmoogra oil were adopted as tentative methods.

<sup>1</sup> Laudolt. *The Optical Rotating Power*, 2nd Ed., p. 498, (111).

(a) *Preparation of Sample.—Tentative.*

If the sample is solid or contains solid particles, warm it until the solids have melted. Shake the entire sample until thoroughly mixed and allow it to cool to room temperature.

(b) *Solubility in Alcohol.—Tentative.*

To 25 cc. of the oil in a suitable graduated apparatus (a Rose and Hertzfeld fusel oil apparatus is very satisfactory), add 100 cc. of alcohol and shake thoroughly for 15 minutes. Allow the mixture to stand overnight and observe the reading of the lower layer. Calculate the percentage by volume of oil dissolved by the alcohol.

(c) *Examination of Alcohol-Soluble Portion.—Tentative.*

Transfer the mixture obtained above to a separatory funnel, drain off the oily layer, and evaporate the alcoholic solution on a steam bath to constant weight. Determine the acid number and the iodine number of the residue (U. S. P. X, 1926, pp. 427, 445).

(d) *Viscosity.—Tentative.*

Determine viscosity as directed in U. S. P. X, pp. 466-7, preferably by the Engler or Saybolt apparatus.

**METHYLENE BLUE.**

The tentative method for the assay of methylene blue (p. 392) was made official (first action).

**PHENOLPHTHALEIN IN TABLETS.**

(1) The tentative iodine method for the determination of phenolphthalein in tablets (p. 400) was made official (final action).

(2) The tentative ether extraction method for the determination of phenolphthalein in tablets (p. 401) was made official (final action).

**PYRAMIDON.**

The four tentative qualitative tests for pyramidon (p. 402) were made official (final action).

**SEPARATION OF QUININE AND STRYCHNINE.**

The following modified Simmond's method for the separation and determination of quinine and strychnine in mixtures was adopted as a tentative method.

Make 50 cc. of the solution acid with citric acid, add an equal volume of water, evaporate to nearly the original volume to remove excess alcohol, cool, and extract with two 15 cc. portions of ether to remove oily material. Make the aqueous solution alkaline with ammonium hydroxide and extract the mixed alkaloids in the usual way with a mixture of two parts of chloroform and one part of ether, using 25, 20, 15, 10, and 5 cc. portions. Evaporate the chloroform and ether in a weighed Erlenmeyer flask or beaker to dryness on a steam bath. Add a little ether and again evaporate to dryness to remove the last traces of chloroform. Dry at 100°C. for 1 hour and weigh to obtain the approximate weight of mixed alkaloids.

Dissolve the alkaloidal residue in 50 cc. of 10 per cent sulfuric acid, add 5 cc. of 4 per cent potassium ferrocyanide drop by drop from a buret, stirring well, and set aside

for a few hours or overnight. Filter the resulting precipitate through a small (7 cm.) filter and wash three times with 3 cc. of 5 per cent sulfuric acid. Reserve the filtrate for the determination of quinine. Wash the precipitate immediately into a small separatory funnel with water, transferring the precipitate remaining in the flask to the separatory funnel by shaking about three times with 3 cc. of ammonium hydroxide and a small quantity of chloroform. Extract the ammoniacal solution of the precipitate with 25, 15, 15, 10, and 5 cc. portions of chloroform. Collect the chloroform solutions in another separatory funnel and extract the alkaloids by shaking with 25, 10, 10, and 5 cc. portions of 20 per cent sulfuric acid; repeat the precipitation with potassium ferrocyanide and the other operations, as above, until the chloroform extracts are again obtained, reserving the filtrate for determination of quinine. Evaporate the chloroform carefully, adding a little alcohol toward the end to prevent sputtering. Weigh the residue of strychnine after drying it for one hour at 100°C. This residue should be nearly white and free from quinine. Check volumetrically as follows: Dissolve the residue in hot alcohol, add 0.02 *N* sulfuric acid until the solution is acid to methyl red indicator [p. 396, 52 (C)], then add 2 or 3 cc. in excess. Evaporate most of the alcohol, cool, and titrate back with 0.02 *N* alkali. One cc. of 0.02 *N* acid is equivalent to 6.69 mg. of strychnine ( $C_{21}H_{22}O_2N_2$ ) or 8.57 mg. of strychnine sulfate  $[(C_{21}H_{22}O_2N_2)_2 H_2SO_4 \cdot 5H_2O]$ .

Combine the two filtrates from the precipitations with potassium ferrocyanide in a separatory funnel; make alkaline with ammonium hydroxide; and extract with a mixture of two parts of chloroform and one part of ether, using 20, 15, 15, 10, and 5 cc. portions of the solvent and observing the usual precaution of washing the stem of the delivery tube with the chloroform-ether mixture after each extraction. Wash the combined extractions in a second separatory funnel with two 5 cc. portions of water, transfer to a weighed beaker, evaporate to dryness, add a few cc. of ether, and again evaporate to dryness to remove the final traces of chloroform; dry at 120°–130°C., cool, and weigh as anhydrous quinine. Test the residue qualitatively<sup>1</sup> for quinine or if desired, check the quantity volumetrically as follows: Dissolve the residue in a little alcohol, add 7 drops of methyl red indicator [p. 396, 52 (C)], then add 0.02 *N* sulfuric acid to a distinct red, and 1 cc. in excess. Evaporate the solution to a small volume, cool, allow the quinine sulfate to separate, filter through a small pledget of cotton in the stem of a funnel, wash with small portions of water, and titrate the combined filtrate and washings with 0.02 *N* alkali. One cc. of 0.02 *N* sulfuric acid is equivalent to 6.486 mg. of anhydrous quinine ( $C_{20}H_{24}O_2N_2$ ); to 7.567 mg. of quinine alkaloid ( $C_{20}H_{24}O_2N_2 \cdot 3H_2O$ ); to 7.467 mg. of quinine sulfate, anhydrous,  $[(C_{20}H_{24}O_2N_2)_2 H_2SO_4]$ ; and to 8.728 mg. of quinine sulfate  $[(C_{20}H_{24}O_2N_2)_2 H_2SO_4 \cdot 7H_2O]$ .

#### SILVER PROTEINATES.

(1) The following method for the determination of total silver in silver proteinate<sup>2</sup> was made official (first action).

#### METHOD FOR TOTAL SILVER.

Place 1 gram, accurately weighed, in a 500 cc. Kjeldahl flask. Add 15 cc. of concentrated sulfuric acid and then 10 cc. of concentrated nitric acid. Place on a steam bath for a few minutes, with occasional rotation, to insure a homogeneous mixture. Boil to white fumes. Add more nitric acid, boil again to a clear colorless solution, and cool. Add 100 cc. of distilled water and boil until free of nitrogen oxides. Cool, dilute to 300 cc., add 5 cc. of nitric acid and 5 cc. of ferric ammonium sulfate test solution, and titrate with 0.1 *N* potassium sulfocyanate.

<sup>1</sup> U. S. P. X, 1926, 312.

<sup>2</sup> *This Journal*, 1925, 8: 551.

Number of cc. of 0.1 *N* potassium sulfocyanate  $\times 0.010788 \times 100$  = percentage by weight of silver.

(2) The following method for detection and estimation of ionizable silver compounds<sup>1</sup> was made official (first action).

#### DETECTION AND ESTIMATION OF IONIZABLE SILVER COMPOUNDS.

Weigh a strip of commercial dialyzing tubing 55 mm. wide and about 1 foot long. Wet with distilled water until uniformly pliable. Shake free of adhering water and partially dry by rolling in a clean paper towel. Reweigh while still moist and place in a 250 cc. beaker. (Sheets of dialyzing parchment paper may be used in place of tubing. Fold a square piece of sufficient size over one end of a glass tube, 1 inch  $\times$  4 inches, and secure it in place with a rubber band. This insures a container of the proper size. Dialyzing material should be kept in a humid container to prevent breaking when handled.) Weigh 1 gram of the sample and dissolve it in 15 cc. of water; transfer to the dialyzing tube. Calculate, and add sufficient water to the beaker to make a total of 100 cc. (This insures 20 cc. in the dialyzing tube and 80 cc. in the beaker.) Adjust the tubing to form a "U" in the beaker, cover with a watch glass, and place in a cool dark closet for 24 hours.

#### *Qualitative Test.*

Test a few cc. of the clear colorless solution from the beaker for silver ions by the addition of a few drops of dilute hydrochloric acid and a trace of nitric acid.

#### *Quantitative Method.*

If silver ions are present, remove 50 cc. of the clear colorless solution from the beaker (representing 0.5 gram of sample), dilute to 100 cc., and add 2 cc. of ferric ammonium sulfate and the same quantity of colorless concentrated nitric acid. Titrate with 0.01 *N* potassium sulfocyanate volumetric solution and calculate to percentage by weight of the silver (ionizable):

1 cc. of 0.001 *N* potassium sulfocyanate is equivalent to 0.0010788 gram of silver.

#### TURPENTINE OIL.

The tentative sulfuric-fuming nitric acid method for the determination of mineral oil in turpentine (p. 409) was modified to read as follows:

Place 50 cc. of the turpentine in a 300 cc. Kjeldahl or other long-necked flask, cool in ice water, and add slowly with constant agitation, 25 cc. of concentrated sulfuric acid. Shake well to obtain complete reaction, keeping the flask cool. When the reaction is complete, cool thoroughly, and add 25 cc. of water. Distil the polymerized mixture in a current of steam, collecting 500 cc. of total distillate. Separate the oil from the aqueous portions.

Place a volume of fuming (sp. gr. 1.5) nitric acid equal to three times the volume of the oil in a 200-250 cc. separatory funnel and cool in ice water. Add the oil cautiously drop by drop, shaking carefully and keeping the mixture cool. After all the oil has been added, allow the funnel to stand quietly, very lightly stoppered, about 30 seconds, until the oil has a chance to come to the surface. Then draw off the acid and wash the remaining oil once with a little fuming nitric acid, once with strong nitric acid, and finally several times with water. Measure the volume of the oil, record its consistency and color, and determine its refractive index at 20°C. Pure gum spirits of turpentine gives less than 0.5 per cent residue by this method.

<sup>1</sup> *This Journal*, 1925, 8: 551.



## XXXII. REFERENCE TABLES.

No additions, deletions, or other changes.

## EGGS AND EGG PRODUCTS.

The following methods for the examination of eggs and egg products were adopted at the 1924 and 1925 meetings:

COLLECTION AND PREPARATION OF SAMPLE.<sup>1</sup>—TENTATIVE.

No simple rules can be made for the collection of a sample representative of the average of any particular lot of egg material as the conditions encountered may differ widely. Experienced judgment must be called upon in each instance. Generally speaking, where large lots are under examination, it is best to draw a number of samples for separate analyses rather than to attempt to get one composite representative sample.

(a) *Liquid Eggs*: Secure representative container or containers. Mix the contents of a container thoroughly and draw about a 300 gram sample for analysis. A long handled dipper or ladle serves well to withdraw the sample. Keep the sample hermetically sealed in a jar in a cool place. Report odor and appearance and examine for foreign material.

(b) *Frozen Eggs*: Secure representative container or containers. Examine contents as to odor and appearance and for foreign material. The condition of the contents can be determined best by boring a hole to the center of a container with an auger and noting the odor as the auger is withdrawn. If impossible to secure individual containers, samples may consist of the composite of the borings made on the contents of each container. Borings should be taken midway between the center and circumference of the top of the can from at least three widely separated parts and should extend to as near the bottom of the can as possible. Collect about 300 grams of the sample. Keep hermetically sealed in a jar in a cool place and in a frozen state if possible. When ready for analysis, warm the samples in a bath held below 50°C. and mix well.

(c) *Powdered Dried Eggs*: Secure representative container or containers. For small packages, take entire parcel or parcels for the sample. For boxes and barrels, remove the top layer to a depth of about 6 inches with a flour scoop or other convenient instrument. Draw small quantities of sample totaling about 300–500 grams from accessible parts of the container and place in a hermetically sealed jar. Report odor and appearance and examine for foreign material. Prepare the sample for analysis by mixing three times through a domestic flour sifter to assure complete breaking up of lumps. Keep in a hermetically sealed jar in a cool place.

(d) *Flaked and Drum Dried Eggs*.—Collect the sample as directed for powdered dried eggs. Report odor and appearance and examine for foreign material. Prepare albumen samples for analysis by grinding in a mill to pass entirely a 60-mesh sieve, and whole egg and yolk samples to pass entirely a 20-mesh sieve or as fine as is practicable. Keep in a hermetically sealed jar in a cool place.

## TOTAL SOLIDS.

I. Vacuum Method<sup>2</sup>.—Tentative.

## APPARATUS.

(a) *Metal dish*.—Diameter about 55 mm., height about 15 mm., provided with an inverted slip-in cover fitting tightly on the inside.

<sup>1</sup> This Journal, 1925, 8: 273, 599.

<sup>2</sup> Ibid., 600.

(b) *Air-tight desiccator*.—Should contain reignited quick lime or calcium carbide.

(c) *Vacuum oven*.—Should be connected with a pump capable of maintaining a partial vacuum in the oven with a pressure equivalent to 25 mm. or less of mercury and provided with a thermometer passing into the oven in such a way that the bulb is near the samples. A concentrated sulfuric acid gas drying bottle is connected with the oven for admitting dry air for releasing the vacuum.

(d) *Mercury manometer*.—Used to indicate the pressure of the partial vacuum.

#### DETERMINATION.

(a) *Dried eggs*.—Weigh accurately about 2 grams of the well-mixed sample in a covered dish that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after attaining room temperature. Loosen the cover (do not remove) and heat at 98°–100°C. to constant weight (approximately 5 hours) in a partial vacuum having a pressure equivalent to 25 mm. or less of mercury. Admit dry air into the oven to bring to atmospheric pressure. Immediately tighten the cover on the dish, transfer to the desiccator, and weigh soon after room temperature is attained. Report the weight of egg residue as total solids.

(b) *Liquid or frozen eggs*.—Weigh accurately about 5 grams of the sample in a covered dish that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after attaining room temperature. Remove the cover and drive off most of the water by heating on a steam bath. Replace the cover loosely and complete the drying in a partial vacuum as directed under dried eggs (a).

#### II. Routine Air-Oven Method.—Tentative.

(This method gives results closely approximating those by the vacuum method.)

#### APPARATUS.

(a) *Metal dish and desiccator*.—Same as for the vacuum method.

(b) *Drying oven*.—Should maintain a temperature of 112°–117°C. and be provided with an opening for ventilation and with a thermometer passing into the oven in such a way that the bulb is near the samples.

#### DETERMINATION.

*Liquid egg*.—Weigh accurately about 5 grams of homogeneous sample in the covered dish that previously has been dried at 112°–117°C., cooled in the desiccator, and weighed soon after attaining room temperature. Remove the cover and drive off most of the water by heating on a steam bath for approximately 30 minutes. Dry the dish, cover, and contents in the oven at 112°–117°C. for approximately 3 hours. Cover the dish while still in the oven, transfer to the desiccator, and weigh soon after room temperature is attained. Report the egg residue as total solids.

*Powdered dried egg*.—Use approximately 2 grams of the finely powdered, well mixed sample accurately weighed. Follow the directions for liquid eggs, omitting the preliminary drying on a steam bath. Dry for approximately one hour. Report the egg residue as total solids.

#### ORGANIC AND AMMONIACAL NITROGEN.<sup>1</sup>—OFFICIAL.

*Powdered dried eggs*: Transfer about 1 gram of the well mixed sample accurately weighed to a 500 cc., or preferably 800 cc., kjeldahl flask. Continue as directed below.

*Liquid eggs*: Weigh 2–3 grams of well mixed sample by difference into a 500 cc., or preferably an 800 cc., Kjeldahl flask. Continue as follows:

<sup>1</sup> *This Journal*, 1925, 8: 273, 801.

Determine the nitrogen as directed on p. 7, 19, or p. 8, 22 or 24. (Complete digestion of the sample is accomplished most rapidly by the Kjeldahl-Gunning-Arnold method.) Distil the ammonia into 30–50 cc. of 0.1 *N* standard acid.

#### FAT (ACID HYDROLYSIS METHOD).<sup>1</sup>—TENTATIVE.

##### PREPARATION OF SOLUTION.

*Liquid eggs.*—Weigh accurately by difference approximately 5 grams of the well mixed sample into a 50 cc. beaker. Add 10 cc. of strong hydrochloric acid, mix well, set the beaker in a water bath held at 75°–80°C., and stir at frequent intervals for 15–25 minutes, or until the sample is sufficiently hydrolyzed to form a clear solution. Add 10 cc. of 95 per cent alcohol and cool.

*Powdered dried eggs.*—Weigh accurately 2 grams of the well mixed sample into a 50 cc. beaker, add 2 cc. of 95 per cent alcohol, and stir to moisten all particles (the moistening of the sample with alcohol prevents lumping on addition of the acid). Add 10 cc. of hydrochloric acid (sp. gr. 1.125, or 25 + 13), mix well, set the beaker in a water bath held at 75°–80°C., and stir at frequent intervals for 15–25 minutes, or until the sample is sufficiently hydrolyzed to form a clear solution. Add 10 cc. of 95 per cent alcohol and cool.

##### DETERMINATION.

Transfer the mixture to a Röhrig or Mojonner fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc. of ethyl ether in three portions and shake the mixture well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.) and mix well. Let stand until the ether layer is practically clear. Through a filter consisting of a pledget of cotton packed just firm enough in the stem of a funnel to allow free passage of the ether, draw off as much as possible of the ether-fat solution into a weighed 125 cc. beaker-flask containing some porcelain chips. Before weighing the beaker-flask, dry it in an oven at the temperature of boiling water and then allow it to stand in the air to constant weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc. of each ether. Shake well on the addition of each ether. Draw off the clear ether solutions through the filter into the same flask as before and wash the tip of the spigot, the funnel, and the end of the funnel stem with a small quantity of a mixture of the two ethers in equal parts free from suspended water. Evaporate the ethers slowly on a steam bath, then dry the fat in a boiling water oven to constant weight (approximately 90 minutes). Remove the fat-flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. Correct this weight by a blank determination on the reagents used.

#### LIPOIDS AND LIPOID PHOSPHORIC ACID<sup>2</sup> (P<sub>2</sub>O<sub>5</sub>).

##### LIPOIDS.—TENTATIVE.

(a) *Liquid eggs.*—Weigh accurately by difference approximately 10 grams of the well mixed sample into a 200 cc. nursing bottle, add 100 cc. of anhydrous ether, stopper with a softened cork, and shake vigorously. Add five 5 cc. portions of 95 per cent alcohol and shake after each addition. (The gradual addition of alcohol with shaking coagulates the proteins in a very fine state.) Centrifugalize and decant the liquid into a 250 cc. beaker containing some bits of broken porcelain. Wash the neck of the bottle with ether, and place the beaker with the fat solution on a steam bath. Add 15 cc. of 95 per cent alcohol to the egg residue in the bottle in such a way as to wash down any particles adhering to the sides and set in a water bath held at 70°–80°C. for 15 minutes. Shake occasionally with a rotary motion so as to moisten all particles with the alcohol.

<sup>1</sup> *This Journal*, 1925, 8: 273, 601.

<sup>2</sup> *Ibid.*, 273, 602.

Cool, add 30 cc. of ether, stopper, shake for 5 minutes, centrifugalize to throw down suspended particles, and decant the liquid into the original 250 cc. beaker. Rinse the bottle neck with ether. Re-extract the residue with two successive 20 cc. portions of ether, shake 1 minute each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts to just dryness on a steam bath. Drive off any remaining apparent moisture on the sides of beaker by placing in a boiling water oven for about 5 minutes. Dissolve the dried extract in about 15 cc. of chloroform and filter the solution into a previously dried and weighed flat-bottomed platinum dish through a pledget of cotton packed in the stem of a funnel. Free any solid extract adhering to the beaker with a glass rod and transfer through the filter into the platinum dish by means of chloroform from a wash-bottle all soluble extract from the beaker bottom and sides. Finally wash the funnel and stem tip. (The filtrate should be perfectly clear.) Evaporate the chloroform on a steam bath (an electric fan may be used to hasten evaporation) and dry the dish and contents in a boiling water oven to constant weight (approximately 90 minutes). Weigh, and report the extract as lipoids.

(b) *Powdered dried egg*.—Transfer about 2 grams of well mixed sample, accurately weighed, to a funnel having a pledget of cotton loosely placed in the stem. Wash with ether four or five times to extract most of the ether-soluble substances. Collect the washings in a 250 cc. beaker containing some bits of broken porcelain and place on a steam bath. Transfer the residue and cotton in the funnel to a small glass mortar and allow the ether to evaporate at room temperature. Add 2–3 grams of precipitated calcium carbonate to the egg residue, grind to a fine powder, and transfer all to a 200 cc. nursing bottle. Wash the mortar, pestle, funnel, and funnel-stem tip with ether and add washings to the original ether extract. Continue as directed under *Liquid Eggs*, beginning with “Add 15 cc. of 95 per cent alcohol to the egg residue in the bottle”.

#### LIPOID PHOSPHORIC ACID ( $P_2O_5$ ) -- TENTATIVE.

Dissolve the lipoids in 10–15 cc. of chloroform, add 10–20 cc. of 4 per cent alcoholic potassium hydroxide solution, evaporate to dryness on a steam bath, and char completely in a furnace at a faint red heat. Cover the dish with a cover glass, add sufficient dilute nitric acid (1 + 3) to make the solution slightly acid, and filter into a 100 cc. volumetric flask. Wash the filter and residue carefully, make up the filtrate to 100 cc., and determine the phosphoric acid as directed on p. 3, 7 or 10. For the volumetric method pipet 20 cc. of the solution into a 250 cc. beaker, neutralize with dilute ammonium hydroxide (1 + 3), and then slightly acidify with dilute nitric acid (1 + 3). Set the beaker in a water bath held at 45°–50°C. and add 15 grams of ammonium nitrate. When the solution has reached the temperature of the bath, add sufficient ammonium molybdate solution, previously heated to 45°–50°C., to precipitate all the phosphates; stir; and heat for 30 minutes. Filter the precipitate on an asbestos mat in a Hirsch funnel, wash with cold water, and proceed as directed on p. 4, 10 (a), beginning with “Transfer the precipitate and filter to the beaker or precipitating vessel”. Report as lipoid phosphoric acid ( $P_2O_5$ ).

#### REPORT OF THE BOARD OF EDITORS.

By R. W. BALCOM (Bureau of Chemistry, Washington, D. C.), *Chairman*.

The report of the Board of Editors of *The Journal* this year is brief and of necessity rather general in character. The two recommendations made last year and approved by the association through its Executive

Committee were put into effect as soon as practicable after the meeting in 1924. The first of these recommendations was that 50 reprints without covers should be furnished free of charge to authors of papers appearing in the "Contributed Papers" section of *The Journal*. The net cost to the association of this service amounted to \$89.61 for the four numbers that have been issued during the association year, namely, the issue of November 15, 1924, and those of February, May, and August, 1925. In the judgment of the board, this sum was well spent, because since the adoption of this policy no complaints have been received, and without doubt the fact that these reprints would be available has been of material assistance in securing for publication some of the papers that have appeared during the year. These contributed papers have added much to the value of *The Journal*.

The second recommendation, namely, that the volume numbers of *The Journal* should be made to coincide with the calendar year, was brought about by publishing the issues of August and November of this year as extra numbers, 5 and 6 of Volume VIII, rather than as numbers 1 and 2 of Volume IX, as would have been the case if the old order had been followed. No criticism of this change has been heard. The subscribers that have referred to the matter have expressed only approval. In billing for renewals a circular letter explaining as clearly as possible the purpose of this change and how it was to be brought about was sent with each bill. No doubt this explanation accounts for the fact that the change has occasioned far less confusion in the handling of subscriptions than was anticipated. The four numbers of Volume IX of *The Journal* will be the issues of February, May, August, and November, 1926, and the complete proceedings of the present meeting of the association will appear in those four numbers.

Another improvement that may be mentioned is the result of a suggestion made by one of the members of the association. This is the footnote appearing in connection with the recommendations made by referees or associate referees at the conclusion of their reports to refer to the page upon which may be found the report of the sub-committee to which these recommendations were referred and of the action of the association thereon.

To the members of the association probably the most interesting part of this report will be that dealing with financial matters pertaining to the two publications of the association, *The Journal* and *Methods of Analysis*. *Methods of Analysis* is distributed through the editorial office, and for that reason the two publications are considered together. During the year expenses aggregating about \$13,000 have been incurred. Of this amount, approximately \$4,500 was for the printing of *The Journal*; the remainder is the expense so far incurred in connection with the new edition of *Methods of Analysis* brought

out during the year. Three thousand copies of the new edition have been printed, and 2025 of these have been bound. As soon as needed the remaining 975 copies will be bound. It is impossible to state just how long this supply will last, but it is certain that a second printing will be necessary before the third edition is issued four or five years hence. In this second printing it is planned to have at least two thousand additional copies run off. Including a first and second printing, approximately 4500 copies of the 1920 edition were issued, and that edition was completely exhausted early in 1924.

It is believed that the sale of the present edition will equal, if it does not exceed, that of the 1920 edition. About 1300 copies have been sold since the first of June, when distribution began, and while sales are now probably at their maximum the first demand has as yet not been satisfied and undoubtedly will continue for several months to come.

Receipts from subscriptions and from advertisements again have been about sufficient to pay the cost of printing *The Journal*. Nearly half of the cost so far incurred in connection with the new edition of *Methods of Analysis* has already been paid. It is believed that it is safe to predict that by the time of the next meeting the remainder of this bill will have been paid, and it is quite possible that at that time the board will be able to report that the association has some surplus to its credit.

The detailed financial statement of receipts and disbursements by the board from October 1, 1924, to October 15, 1925, is appended.

Approved.

# FINANCIAL REPORT ON PUBLICATIONS FROM By R. W. BALCOM (Bureau of Chemistry, RECEIPTS.

*Methods of Analysis.*

Number	Price each	Total
88	\$4.00	\$352.00
41	4.40	180.40
728	5.00	3,640.00
25	5.50	137.50
		<hr/>
		\$4,309.90
Plus gain on exchange		.77
Plus payment for one copy from bankruptcy sale, Pearl Hominy Co.....		.21*
		<hr/>
Total receipts . . . . .		\$4,310.88

*Journal subscriptions.*

Number	Price each	Total
6	\$8.25	\$49.50
330	7.50	2,475.00
2	7.00	14.00
13	6.60	85.80
107	6.00	642.00
3	5.50	16.50
94	5.00	470.00
31	4.40	136.40
47	4.00	188.00
4	2.75	11.00
6	2.50	15.00
5	4.50	22.50
10	2.20	22.00
31	2.00	62.00
7	1.50	10.50
1	1.00	1.00
		<hr/>
Total . . . . .		\$4,221.20
Plus gain on exchange . . . . .		.84
		<hr/>
Total.. . . .		4,222.04

*Advertisements.*

Number	Price each	Total
10	\$25.00	\$250.00
1	15.00	15.00
1	5.00	5.00
		<hr/>
Total . . . . .		270.00

*Reprints.*

Hertwig . . . . .	\$ .50	
Sterling . . . . .	1.00	
Spencer . . . . .	1.50	
Markley . . . . .	6.00	
Needham and Fellers . . . . .	11.50	
Lundell and Hoffman . . . . .	4.00	
† University of Tennessee (old account) . . . . .	9.07	
		<hr/>
		33.57
Total for Journal, Methods, Ads, Reprints . . . . .		\$8,836.49
Plus bank balance of October 1, 1924 . . . . .		278.50
		<hr/>
		\$9,114.99

\* Old account.

† Duplicate payment on old account, credit given University of Tennessee, \$9.07.

OCTOBER 1, 1924, to OCTOBER 15, 1925.

Washington, D. C.), *Chairman, Board of Editors.*

## DISBURSEMENTS.

1924		Amount	Check No.
Dec. 30	Postmaster, Washington, D. C., box rent for quarter ending 3-31-25 .....	\$2.00	100
1925			
Jan. 23	Janet K. Smith, office expenses .....	25.00	101
Jan. 24	F. W. Faxon, refund on subscriptions to <i>Journal</i> .....	18.30	102
Jan. 26	A. O. A. C. dues for University of Tennessee .....	5.00	103
	(See letter of 1-22-25.)		
Feb. 6	Industrial Printing Co., bill of 11-24-24 .....	970.05	104
Feb. 6	Industrial Printing Co., bill of 11-29-24 .....	20.90	105
Feb. 25	J. T. Keister, back numbers of <i>Journal</i> .....	1.50	106
Feb. 26	Postmaster, Washington, D. C., 5,000 window envelopes .....	119.30	107
Mar. 23	Postmaster, Washington, D. C., box rent, quarter ending 6-30-25 .....	2.00	108
Mar. 23	R. P. Andrews Paper Co., bills of Mar. 5-7, 1925 .....	14.78	109
Mar. 26	W. Heffer & Sons, Ltd., reimbursement for overpayment on Vol. 8 .....	3.30	110
Mar. 26	Sun Maid Raisin Growers, reimbursement for overpayment on <i>Methods</i> .....	1.00	111
	Cancelled .....		112
Apr. 13	The Colonial Printery, bill of 4-10-25 .....	30.64	113
Apr. 13	Industrial Printing Co., bill of 3-23-25 .....	12.75	114
Apr. 13	Industrial Printing Co., bill of 3-31-25 .....	30.15	115
Apr. 16	Buckbee Mears Co., bills of Nov. 29 and Dec. 17, 1925 ..	8.51	116
Apr. 16	Janet K. Smith, office expenses .....	25.00	117
Apr. 22	Williams and Wilkins Co., bill of 4-18-25 .....	6.00	118
May 12	Industrial Printing Co., on account bill of 2-28-25 .....	600.00	119
May 25	The Colonial Printery, bills of 4-27-25 and 5-23-25 .....	43.85	120
May 27	Janet K. Smith, office expenses .....	50.00	121
June 26	Postmaster, Washington, D. C., box rent, quarter ending 9-30-25 .....	2.00	122
June 26	Felton & Sons, Inc., back numbers of <i>Journal</i> .....	8.25	123
July 1	Regina Keliher, office expenses .....	50.00	124
July 8	R. P. Andrews Paper Co., bill of 5-30-25 .....	9.60	125
July 8	Industrial Printing Co., bill of 6-25-25 .....	76.05	126
July 8	Industrial Printing Co., balance on bill of 2-28-25 .....	404.13	127
July 20	Industrial Printing Co., on account, bill of 6-25-25 ..	1,211.45	128
July 23	Estelle L. Milne, office expenses .....	50.00	129
July 23	The Colonial Printing Co., bill of 7-14-25 .....	8.00	130
Aug. 10	Industrial Printing Co., bill of 6-30-25 .....	1,164.38	131
Aug. 10	Industrial Printing Co., bill of 7-10-25 .....	18.50	132
Aug. 10	Industrial Printing Co., bill of 7-22-25 .....	36.50	133
Aug. 17	Industrial Printing Co., on account, bill of 7-13-25 .....	1,000.00	134
Aug. 18	Post Office, Washington, D. C., mailing <i>Journals</i> .....	25.00	135
Aug. 21	Estelle L. Milne, office expenses .....	50.00	136
Aug. 22	E. K. Nelson, back numbers, Vol. 1 of <i>Journal</i> .....	4.00	137
Sept. 4	Trustees Canton Christian College, refund on 3 foreign orders, <i>Methods</i> .....	3.30	138
Sept. 9	Industrial Printing Co., on account, bill of 7-13-25 .....	1,000.00	139
Sept. 11	Estelle L. Milne, office expenses .....	50.00	140
Sept. 17	N. A. Fiber Products Co., refund on purchase of 1 copy of <i>Journal</i> (\$5 rec'd) .....	3.50	141
Sept. 21	Post Office, Washington, D. C., quarter ending 12-31-25 ..	2.00	142
Sept. 28	F. W. Faxon Co., refund on subscriptions to <i>Journal</i> ..	6.00	143
Sept. 28	Moore Cottrell Subscription Agency, refund on subscription, foreign .....	4.40	144
Sept. 30	Williams and Wilkins Co., back numbers of <i>Journal</i> .....	2.50	145
Oct. 10	W. W. Skinner, return of loan to office in Dr. Balcom's absence .....	50.00	146
Oct. 10	Industrial Printing Co., bill of 8-20-25 .....	1,160.73	146A
Oct. 10	Industrial Printing Co., bill of 9-29-25 .....	32.56	147
Oct. 10	Industrial Printing Co., balance on bill of 6-25-25 .....	120.00	148
	Plus bank balance, October 15, 1925 .....	571.61	
		<b>\$9,114.99</b>	



## FINANCIAL REPORT OF THE SECRETARY-TREASURER

By W. W. SKINNER (Bureau of

## RECEIPTS.

1924			
Oct.	1	Bank balance.....	\$685.54
		1924 dues received too late for inclusion in 1924 report,	
		3 at \$5.00.....	\$15.00
		1925 dues from institutional members, 69 at \$5.00.....	345.00
1925			
Oct.	10	Reimbursement from <i>Journal</i> account for loan of June 10	50.00
			<hr/>
			410.00

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Total..... \$1,095.54

FROM OCTOBER 1, 1924, TO OCTOBER 15, 1925.

Chemistry, Washington, D. C.).

# DISBURSEMENTS.

		Amount	Check No.
1924			
Oct. 17	Janet K. Smith, reimbursement for expenses, 1924 meeting	\$25.00	36
Oct. 17	Bastian Bros., badges, bill of 10-14-24	26.08	37
Oct. 27	Colonial Printery, registration cards, bill of 10-25-24	5.00	38
Dec. 18	Colonial Printery, bill of 12-12-24	3.50	39
1925			
Jan. 5	Janet K. Smith, office expenses	5.00	40
Jan. 26	N. Y. Farms and Markets, change of dues to subscription	5.00	41
Apr. 6	Pennsylvania State College, change of dues to subscription	5.00	42
June 10	Marian E. Lapp, advance money for office expense in absence of R. W. Balcom	50.00	43
June 15	Marian E. Lapp, mailing programs, 1925	25.00	44
Oct. 10	Industrial Printing Co., programs, bill of 8-19-25	37.00	45
Oct. 15	Bank balance	898.96	
	Checks deposited but payment declined	10.00	

Total . . . . . \$1,095.54

No report was made by the Committee on Quartz Plate Standardization and Normal Weight.

*C. A. Browne:* Mr. Bates has not called any meeting of the committee. Dr. Zerban and I are also on the committee, and we have some work under way at the present time. We hope to have something to report by the time of the next meeting.

## REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS.

The committee recommends the following definitions and interpretation of terms:

### *For Final Adoption as Official.*

#### 1. BASIC PHOSPHATE SLAG.

*Basic phosphate slag* is a by-product in the manufacture of steel from phosphatic iron ores. The product shall be finely ground and shall contain no admixture of materials other than what results in the original process of manufacture. It shall contain not less than twelve per cent (12%) of total phosphoric acid ( $P_2O_5$ ), not less than eighty per cent (80%) of which shall be soluble in two per cent (2%) citric acid solution according to the Wagner method of analysis. Any other phosphate slag not conforming to this definition shall be designated *low grade*.

#### 2. INTERPRETATION OF THE WORD "LIME" AS APPLIED TO FERTILIZERS.

The term *lime* shall not be used in the registration, labelling, or guaranteeing of fertilizers or fertilizing materials, unless the lime is in a form to neutralize soil acidity, such as the oxide, hydroxide, or carbonate, or equivalent magnesia compounds.

#### 3. DRIED PULVERIZED OR SHREDDED MANURES.

*Dried pulverized or shredded manures* shall be only what the name indicates, and not mixtures of manures and other materials.

#### 4. MANURE SALTS.

*Manure salts* shall be understood to mean potash salts containing high percentages of chloride and from twenty per cent (20%) to thirty per cent (30%) of potash ( $K_2O$ ). The term *double manure salts* should be discontinued.

#### 5. SULFATE OF POTASH-MAGNESIA.

*Sulfate of potash-magnesia* is a potash salt containing not less than twenty-five per cent (25%) of potash ( $K_2O$ ), nor less than twenty-five per cent (25%) of sulfate of magnesia, and not more than two and five-tenths per cent (2.5%) of chlorine.

### *Second Recommendation as Tentative.*

#### 1. FERTILIZER FORMULA.

The term *fertilizer formula* shall be interpreted as expressing the quantity and grade of the crude stock materials used in making a fertilizer mixture. For example: 800 pounds of 16 per cent acid phosphate, 800 pounds of 9-20 tankage, and 400 pounds of sulfate of potash-magnesia.

## 2. ANALYSIS.

The word *analysis*, as applied to fertilizers, shall designate the percentage composition of the product expressed in terms of nitrogen or ammonia, phosphoric acid, and potash in their various forms.

## 3. BRAND AND BRAND NAME.

A *brand* is a term, design, or trade mark used in connection with one or several grades of fertilizers.

A *brand name* is a specific designation applied to an individual fertilizer.

## 4. UNIT.

A *unit* of plant food is twenty (20) pounds, or one per cent (1%) of a ton of fertilizer.

## 5. UNLEACHED WOOD ASHES.

*Unleached wood ashes* are defined as ashes resulting from burning unleached wood and that have not had any part of their plant food extracted by contact with water or other solvent.

## 6. LEACHED WOOD ASHES.

*Leached wood ashes* are defined as ashes resulting from burning unleached wood, but as having had part of their plant food removed by artificial means or by exposure to rains, snows, or other solvent.

## 7. ASHES FROM LEACHED WOOD.

*Ashes from leached wood* are defined as unleached ashes resulting from burning wood that has been exposed to or digested in water or other liquid solvents, as in the extraction of dyes, so that a part of the plant food has been dissolved and removed.

## 8. DISSOLVED BONE.

*Dissolved bone* is defined as a ground bone or bone meal that has been treated with sulfuric acid.

## 9. FORM OF NITROGEN IN CYANAMIDE.

The nitrogen in calcium cyanamide shall be considered as being of organic nature.

### *Amended Tentative Interpretations.*

#### 1. INTERPRETATION OF BRAND NAME TO INCLUDE THE ANALYSIS OR GRADE OF FERTILIZER.

The committee recommends and urges the practice of including the analysis or grade of fertilizer with the brand name, both by the manufacturer on sacks and in printed literature and by the control official in his reports and publications.

#### 2. ACTIVITY OF WATER-INSOLUBLE NITROGEN IN MIXED FERTILIZERS.

The alkaline and neutral permanganate methods distinguish between the better and the poorer sources of water-insoluble nitrogen, and do not show the percentage availability of the material. The available nitrogen of any product can be measured only after carefully conducted vegetation experiments.

(a) The methods shall be used on mixed fertilizers containing water-insoluble nitrogen amounting to three-tenths of one per cent (0.3%) or more of the weight of the material. In the event of a total nitrogen exceeding the minimum guarantee, accompanied by a low activity of the insoluble nitrogen, the over-run *may* be taken into consideration in determining the classification of the water-insoluble nitrogen.

(b) The water-insoluble nitrogen in mixed fertilizers showing an activity below fifty per cent (50%) by the alkaline method and also below eighty per cent (80%) by the neutral method shall be classed as inferior. This necessitates the use of both methods before classifying as inferior.

### *First Recommendation as Tentative.*

#### 1. MEANING OF TERM "FINELY GROUND".

The term "finely ground" in the definition of basic phosphate slag shall refer to actual size of particles as determined by the use of standard sieves, as follows: seventy per cent (70%) or more should pass a 100-, and ninety per cent (90%) or more should pass a 50-mesh sieve.

#### 2. SULFATE OF POTASH.

*Sulfate of potash* is a potash salt containing not less than forty-eight per cent (48%) of potash ( $K_2O$ ) in the form of sulfate and not more than two and one-half per cent (2.5%) of chlorine.

#### 3. MAXIMUM AMOUNT OF CHLORINE PERMISSIBLE IN FERTILIZERS IN WHICH THE POTASH IS CLAIMED AS SULFATE.

The *chlorine* in mixed fertilizers in which the potash is claimed as sulfate shall not exceed three-tenths of one per cent (0.3%) more than what is called for in the minimum potash content based on the definition for sulfate of potash as formulated by the committee. Calculate as follows: 0.05 times the percentage of potash found plus 0.3.

#### 4. MURIATE OF POTASH.

*Muriate of potash* is a potash salt containing not less than fifty per cent (50%) of potash ( $K_2O$ ) in the form of chloride.

#### 5. NITRATE OF POTASH.

*Nitrate of potash* is a salt containing not less than twelve per cent (12%) of nitrogen and forty-four per cent (44%) of potash ( $K_2O$ ).

#### 6. DEFINITION OF PRODUCTS SECURED BY HEATING CALCIUM PHOSPHATE WITH ALKALINE SALTS CONTAINING POTASH.

These products are *not* potassium phosphate. They may be called non-acid phosphates, calcium-potassium phosphates, or by some other name that is not misleading.

#### DETERMINATION OF CHLORINE IN MIXED FERTILIZERS.

The committee recommends that this subject be referred to the Associate Referee on Potash.

The following topics are proposed for further consideration:

1. Definition of nitrate of soda.
2. Significance of the words "blood" and "bone", etc., when used in the brand name of mixed fertilizers.
3. Uniform order and terms in expressing the grade of fertilizers.
4. Use of the term "high grade" prefixed to the brand name of mixed fertilizers.

H. D. HASKINS,	J. W. KELLOGG,
R. N. BRACKETT,	C. H. JONES.
G. S. FRAPS,	

*Committee on Definition of Terms and Interpretation of Results on Fertilizers.*

Approved.

## REPORT OF COMMITTEE ON REVISION OF METHODS OF SOIL ANALYSIS.

Since the personnel of the committee is such that it is difficult for the committee to convene, its work has been conducted through correspondence. The principal topic before the committee is the question of the reaction value of soils, and this problem is now being studied by the Associate Referee on Reaction Value of Soils. Some research work has also been done by the chairman upon the problem of a rapid method for the determination of calcium, magnesium, and phosphorus in soils that carry unusually small amounts of these elements. If it should appear desirable so to do, collaborative assistance may be sought later in the development of such a procedure.

W. H. MACINTIRE,	J. A. BIZZELL,
A. W. BLAIR,	ROBERT STEWART.
A. G. MCCALL,	

*Committee on Revision of Methods of Soil Analysis.*

Approved.

## REPORT OF THE COMMITTEE ON RECOMMENDATIONS OF REFEREES.

Through the reports of the chairmen of Sub-committees A, B, and C, the principal activities of the Committee on Recommendations of Referees have been brought to the attention of the association and acted upon. The committee takes this opportunity of expressing its appreciation to the referees and associate referees for the splendid cooperation given during the past year and to congratulate them upon the excellence of the reports submitted. If in some way arrangements could be effected whereby the reports could be placed in the hands of the individual members of the committee for consideration at least one week before the date of the meeting it would be felt that the ideal had been reached. The necessity for this has been discussed so many times in former reports that the committee feels it has nothing more it can say in this connection, but it is urged that the referees accord the committee the courtesy of submitting reports in time so that they may have the consideration justly expected from the Committee on Recommendations of Referees.

The committee has considered the criticisms regarding multiplicity of methods already brought to the attention of the association by the Committee on Editing Methods of Analysis and has joined with that committee in presenting the resolution to the executive committee for a single official method wherever possible. To carry out this policy further the committee respectfully recommends that between now and the next revision of *Methods of Analysis* the referees and associate referees

study those determinations in their respective chapters in which more than one method is given, with a view to retaining a single official method for each determination wherever this is possible. The adoption of the resolution for a single official method, it is believed, will eliminate an uncertainty that has heretofore existed in the consideration of recommendations of referees and associate referees and enable the committee to carry out more definitely the objects of the association.

The list of referees and associate referees will be found on p. 4.

R. E. DOOLITTLE, *Chairman*.

Approved.

## REPORT OF SUB-COMMITTEE A ON RECOMMENDATIONS OF REFEREES.

By B. B. ROSS (Alabama Polytechnic Institute, Auburn, Ala.), *Chairman*.

[Waters, brine, and salt; insecticides and fungicides; soils and liming materials (reaction value of soils); feeding stuffs (linseed meal—determination of starch in the presence of interfering polysaccharides, stock feed adulteration, determination of moisture in cattle feeds); sugar and sugar products (maple products, starch conversion products, drying, densimetric, and refractometric methods, polariscopic methods, chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, potash); plants (inorganic constituents).]

### WATERS, BRINE, AND SALT.

The referee recommends—

(1) That the official method for the determination of hydrogen sulfide in mineral waters<sup>1</sup> be dropped (final action).

Approved:

(2) That the method for the determination of hydrogen sulfide (see p. 29) presented by the referee at the 1924 meeting<sup>2</sup> be made official (final action) after the following minor changes have been made in the wording:

Under "Reagents" (a) eliminate "0.05 N".

Under "Reagents" omit "(b) 0.05 N sodium hydroxide" and change "(c)", "(d)", and "(e)" to "(b)", "(c)", and "(d)", respectively.

Substitute the following sentences, "Transfer a quantity of the sample to a graduated vessel by means of a siphon and add a few drops of phenolphthalein indicator. If alkaline, add hydrochloric acid reagent (a) until the pink color of the indicator disappears", for the first two sentences under "Procedure".

Second, tenth, and sixteenth lines, p. 333, change "(d) or (e)" to "(c) or (d)".

Eighth line, p. 333, change "0.05 N" to "hydrochloric acid (a)" and delete "or 0.05 N alkali reagent (b)".

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 93.

<sup>2</sup> *This Journal*, 1925, 8: 332.

Twelfth line, p. 333, change "(c)" to "(b)".

Approved.

(3) That the referee for next year study methods for the analysis of salt with particular reference to the determination of ingredients that are added to prevent caking.

Approved.

#### TANNING MATERIALS AND LEATHERS.

No report or recommendations.

#### INSECTICIDES AND FUNGICIDES.

The referee recommends—

(1) That the work on mineral oil-soap emulsions be continued, the methods suggested by the referee being used, and that these methods (see p. 28) be adopted as tentative methods with the view to their adoption as official methods after further collaborative study in 1926.

Approved.

(2) That the xylene distillation method (see p. 28) for water in soaps, given in the referee's report, be adopted as a tentative method with the view to its adoption as an official method after further collaborative study in 1926.

Approved.

(3) That the Kissling method for the determination of nicotine in tobacco and tobacco extract<sup>1</sup> be dropped as an official method (final action).

Approved.

#### SOILS AND LIMING MATERIALS.

##### REACTION VALUE OF SOILS.

It is recommended that the Wherry method of stating results as to the reaction value of soils, or some equally simple method, as recommended by the associate referee and approved by the general referee, be studied with a view to later adoption as a standard.

Approved.

#### FEEDING STUFFS.

##### EXAMINATION OF WHOLE GRAIN.

It is recommended that one or more associate referees be designated to study methods for the examination of whole grain as wheat, corn, oats, barley, rye, etc. The methods should include the collection and preparation of sample and the important methods of analysis. These studies should also include the direction of collaborative work and the preparation of the method in a form for adoption. Where possible these methods should harmonize with the association methods for cereal foods that are of recent development or that are being actively studied.

Approved.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 66.



LINSEED MEAL—DETERMINATION OF STARCH IN THE PRESENCE OF INTERFERING  
POLYSACCHARIDES.

The referee recommends that the tentative method (see p. 31) for the determination of starch in the presence of interfering polysaccharides (linseed meal), as given by the associate referee in his report, be adopted as official (final action).

STOCK FEED ADULTERATION.

The referee recommends—

(1) That the Gensler method (see p. 32) for detection of salt in cattle feeds, as given in the associate referee's report, be adopted as official (first action).

Approved.

(2) That further work be done on the method for determining approximately the amount of oat hulls in oats.

Approved.

MINERAL MIXED FEEDS.

The referee recommends that an associate referee be appointed to study methods for the analysis of mineral mixed feeds during the coming year.

Approved.

DETERMINATION OF MOISTURE IN CATTLE FEEDS.

The referee recommends—

(1) That the proposed distillation method (see p. 30) for the determination of moisture in cattle feeds, as given in the associate referee's report, be adopted as tentative.

Approved.

(2) That the method be given further study with a view to determining its value as a rapid procedure and also its value with materials that do not give reliable results by drying methods.

Approved.

SUGARS AND SUGAR PRODUCTS.

MAPLE PRODUCTS.

The referee recommends that the studies outlined by the Associate Referee on Maple Products in his report for the current year be continued during the coming year.

Approved.

STARCH CONVERSION PRODUCTS.

The referee recommends that a further study be made of the enzyme method for the determination of maltose, as suggested by the associate referee.

Approved.

## DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS.

The referee recommends that work be done in connection with "the establishment of accurate densimetric and refractometric data and tables on fructose and glucose and on the basis of these that there be a critical study made of drying methods starting with pure sugar solution and progressing more gradually toward more complicated mixtures finally resembling the various sugar products".

Approved.

## POLARISCOPIC METHODS.

The associate referee recommends—

(1) That the basic divisor for both the standard and the rapid invertase method, used in the absence of raffinose, be increased from 142.0 to 142.1 and adopted as official (first action).

Approved.

(2) That the methods of polariscopic analysis in the absence of raffinose<sup>1</sup>, presented last year, be adopted by this association as official (final action).

Approved.

(3) That the method of determining sucrose and raffinose in the presence of each other by the two-enzyme procedure of Paine and Balch<sup>2</sup> (see p. 33) be adopted as official (first action).

Approved.

(4) That the investigation presented in the report of the associate referee under A (Determination of sucrose in the absence of raffinose) be continued, invert sugar and reversion products, also other non-sugars occurring in saccharine products not derived from the beet, being used besides sucrose.

Approved.

(5) That the investigation presented under B (Determination of sucrose and raffinose by the two-enzyme method of Paine and Balch) be repeated next year, the quantities of raffinose used being extended beyond this year's limit of 3 per cent.

## CHEMICAL METHODS FOR REDUCING SUGARS.

The associate referee recommends—

(1) That the Lane-Eynon method<sup>3</sup> for the volumetric determination of reducing sugars be adopted as a tentative method (see p. 35).

Approved.

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<sup>1</sup> *This Journal*, 1925, 8: 256.

<sup>2</sup> *Ind. Eng. Chem.*, 1925, 17: 240.

<sup>3</sup> *J. Soc. Chem. Ind.*, 1923, 42: 32 T.

(2) That the tentative volumetric method described in Sections 32 and 33 of Chapter XIII<sup>1</sup> be discarded.

Approved.

(3) That the methods for the electrolytic deposition of copper embodied in Sections 40 and 42 of Chapter XIII<sup>2</sup> be discarded.

Approved.

(4) That the method for the electrolytic deposition of copper from sulfuric and nitric acid solution be rewritten in order to embody the details formerly incorporated in Section 40 (see p. 38).

Approved.

#### FERTILIZERS.

##### PHOSPHORIC ACID.

The referee recommends that work in connection with the gravimetric determination of phosphoric acid, as suggested by the associate referee, be continued during the ensuing year.

Approved.

##### NITROGEN.

The referee recommends—

(1) That, as suggested by the associate referee, the zinc-iron method<sup>3</sup> be placed under the heading "nitrogen in nitrate salts" as it is unsuitable for mixed fertilizers.

Approved.

(2) That collaborative work be done on the Breckenridge method for inorganic nitrogen in mixed fertilizers, as suggested by the associate referee.

Approved.

(3) That, as recommended by the associate referee, further study be made with a view to devising an accurate method for inorganic nitrogen in mixed fertilizers when calcium cyanamide is present.

Approved.

##### POTASH.

The referee recommends—

(1) That the study of the method using magnesium chlorides is not promising and should be discontinued.

Approved.

(2) That further study be made on methods for the prevention of the formation of metaphosphates, as they probably cause errors in the results.

Approved.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 190.

<sup>2</sup> *Ibid.*, 192-3.

<sup>3</sup> *Ibid.*, 11.

## PLANTS.

## INORGANIC CONSTITUENTS OF PLANTS.

The referee recommends that a further study be made during the coming year of the proposed methods for the determination of calcium, iron, and aluminium in plants.

## REPORT OF SUB-COMMITTEE B ON RECOMMENDATIONS OF REFEREES.

By H. C. LYTHGOE (Department of Public Health, Boston, Mass.),  
*Chairman.*

[Testing chemical reagents, spices and condiments, naval stores (turpentine), specific gravity and alcohol, drugs (acetylsalicylic acid, alcohol in drugs, arsenicals, camphor and monobromated camphor, chaulmoogra oil, chloramine, chloroform and carbon tetrachloride, ipecac alkaloids, radio activity in drugs and water, laxative and bitter tonics, mercurials, methylene blue, phenolphthalein, pyramidon, separation of quinine and strychnine, silver proteinates, nitroglycerine, apomorphine, santonin, ether in drug products, barbital and phenobarbital, bio-assay of drugs).]

## TESTING CHEMICAL REAGENTS.

It is recommended that observations of the quality of chemical reagents be continued as heretofore.

Approved.

## SPICES AND CONDIMENTS.

No report was submitted. The committee recommends a repetition of the recommendation of last year relative to salad dressing<sup>1</sup>.

## NAVAL STORES.

No formal report was presented. The committee recommends further study of the subject.

## TURPENTINE OIL (SPIRITS OF TURPENTINE).

It is recommended that the tentative sulfuric-fuming nitric acid method<sup>2</sup> for the determination of mineral oil in turpentine oil be modified as recommended by the referee in his report this year and that the modified method (see p. 55) be further studied with a view to making it official.

## SPECIFIC GRAVITY AND ALCOHOL.

The committee recommends further study of the correlation of refractometric and pycnometric methods for the determination of alcohol.

<sup>1</sup> *This Journal* 1925, 8: 264

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 409.

## DRUGS.

## ACETYLSALICYLIC ACID.

It is recommended—

(1) That the tentative method (Method II, revised) for the determination of combined acetic acid<sup>1</sup> (see p. 49) be made official (first action).  
Approved.

(2) That the tentative method for the determination of acetylsalicylic acid in mixtures containing acetphenetidin and caffeine<sup>1</sup> (see p. 50) be made official (final action).  
Approved.

(3) That the tentative method for the determination of free salicylic acid<sup>2</sup> be made official (final action).  
Approved.

(4) That the bromine method for total salicylates<sup>3</sup> be made official (first action).  
Approved.

(5) That the tentative double titration method for the determination of acetylsalicylic acid<sup>3</sup> (see p. 50) be modified to include the wet extraction method for preparation of sample and the single titration procedure for the final determination as described by the referee in his report this year and that the modified method be made official (first action).  
Approved.

(6) That the tentative qualitative test for free salicylic acid in acetylsalicylic acid<sup>4</sup> be made official (first action).  
Approved.

## ALCOHOL IN DRUGS.

It is recommended that the procedure for the determination of alcohol in drugs, described by the associate referee and amended by the referee, be studied collaboratively during the coming year and that attention be given to the determination of small quantities of alcohol.

Approved.

## ARSENICALS.

It is recommended that the method proposed by the referee for the determination of arsenic in sodium cacodylate (see p. 51) be adopted as tentative and that it be studied next year with a view to making it official. (This method differs in principle from the U. S. P. method.)

Approved.

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<sup>1</sup> *This Journal*, 1925, 8: 506

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 337.

<sup>3</sup> *Ibid.*, 388.

<sup>4</sup> *Ibid.*, 387.

## CAMPHOR AND MONOBROMATED CAMPHOR.

It is recommended—

(1) That the method for the determination of camphor (see p. 52), as described by the referee, be made tentative.

Approved.

(2) That Method I<sup>1</sup>, now tentative, for the determination of monobromated camphor in tablets be made official (first action).

Approved.

## CHAULMOOGRA OIL.

It is recommended—

(1) That the following methods described by the referee last year<sup>2</sup> and not in the U. S. P. X be adopted as tentative: Preparation of sample, solubility in alcohol, examination of alcohol-soluble portion, and viscosity.

Approved.

(2) That further study be given to color reactions of chaulmoogra oil and to studies of loss or gain of weight on heating.

Approved.

## CHLORAMINE.

It is recommended that the study of chloramine be referred to the Referee on Preservatives.

Approved.

## CHLOROFORM AND CARBON TETRACHLORIDE.

It is recommended that further study be given to chloroform and carbon tetrachloride, as outlined by the referee.

Approved.

## IPECAC ALKALOIDS.

It is recommended that the methods for the determination of ipecac alkaloids reported by the referee be studied further in comparison with the methods in the U. S. P. X.

Approved.

## RADIO ACTIVITY IN DRUGS AND WATER.

The committee recommends the continuation of the work as outlined in Recommendation 2 of 1924<sup>3</sup>.

## LAXATIVES AND BITTER TONICS.

It is recommended that work be continued as outlined in 1924<sup>3</sup>.

Approved.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 393.

<sup>2</sup> *This Journal*, 1925, 8: 517. See also p. 52.

<sup>3</sup> *Ibid.*, 267.

## MERCURIALS.

It is recommended that further study be made of methods for the examination of mercurials.

Approved.

## METHYLENE BLUE.

It is recommended that the tentative iodometric assay method for the determination of methylene blue<sup>1</sup> be adopted as official (first action).

Approved.

## PHENOLPHTHALEIN.

It is recommended that the iodometric method and the ether extraction method for the determination of phenolphthalein, now tentative<sup>2</sup>, be made official (final action).

Approved.

## PYRAMIDON.

It is recommended that the four qualitative tests, as published<sup>3</sup>, be made official (final action). Further study is recommended of the present tentative quantitative methods<sup>4</sup> with a view to making them official.

Approved.

## SEPARATION OF QUININE AND STRYCHNINE.

It is recommended that the modified Simmond's method for the separation of quinine and strychnine in mixtures<sup>5</sup> (see p. 53) be adopted as tentative.

Approved.

## SILVER PROTEINATES.

It is recommended—

(1) That the tentative method for total silver<sup>6</sup> (see p. 54), be made official (first action).

Approved.

(2) That the tentative qualitative and quantitative methods for ionizable silver compounds<sup>6</sup> (see p. 55), be made official (first action).

Approved.

(3) That further study be given to the method for ionizable silver by yeast.

Approved.

## NITROGLYCERIN.

It is recommended that collaborative study be given to methods submitted by the referee.

Approved.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 392.

<sup>2</sup> *Ibid.*, 400, 401.

<sup>3</sup> *Ibid.*, 402.

<sup>4</sup> *This Journal*, 1925, 8: 546.

<sup>5</sup> *Ibid.*, 548.

<sup>6</sup> *Ibid.*, 551.

## APOMORPHINE.

It is recommended that collaborative study be given to methods along the lines suggested by the referee.

Approved.

## SANTONIN.

It is recommended that collaborative study be given to the methods outlined by the referee.

Approved.

## ETHER IN DRUG PRODUCTS.

It is recommended that a referee be appointed to study this subject.

Approved.

## BIO-ASSAY OF DRUGS.

It is recommended that the work on the bio-assay of drugs be continued.

Approved.

## BARBITAL AND PHENOBARBITAL.

It is recommended that the tentative method for the estimation of barbital and phenobarbital<sup>1</sup> (see p. 51), be adopted as official (first action).

Approved.

## REPORT OF SUB-COMMITTEE C ON RECOMMENDATIONS OF REFEREES.

By R. E. DOOLITTLE (U. S. Food and Drug Inspection Station, Transportation Building, Chicago, Ill.), *Chairman*.

[Dairy products (cheese, malted milk and dried milk, ice cream), fats and oils, baking powders and baking chemicals (fluorides in baking powder), eggs and egg products, food preservatives, coloring matters in foods, metals in foods, fruits and fruit products (fruit acids, added water in grape juice, ash in fruit products), canned foods, vinegars, flavors and non-alcoholic beverages, meat and meat products (separation of meat proteins, determination of sugar), gelatin, cereal foods (flour, baked cereal products, alimentary pastes, general), cacao products (microscopical methods, crude fiber, cacao butter).]

## DAIRY PRODUCTS.

It is recommended—

(1) That the cryoscopic method<sup>2</sup> for the determination of added water in milk be adopted as an official method (first action) for the determination of added water in cream.

<sup>1</sup> *This Journal*, 1924, 8: 48; 1925, 8: 512.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 265.



The committee does not approve this recommendation but recommends that the method be adopted as a tentative method and submitted to collaborative study during the coming year and that this study include samples of cream containing added water.

Action of committee approved.

(2) That an associate referee be appointed to study methods for the examination of butter, particularly methods for (a) sampling, (b) preparation of sample, (c) acidity, and (d) distinguishing the product made from pasteurized cream.

Approved.

(3) That the methods<sup>1</sup> for the determination of albumin in milk including the suggested use of Almen's reagent for precipitating the albumin be studied during the coming year.

Approved.

(4) The committee further recommends that the Referee on Dairy Products consider during the coming year the suggestions made by H. C. Waterman for the determination of casein in milk.

Approved.

#### CHEESE.

It is recommended—

(1) That the method (see p. 44) for the determination of moisture in cheese by drying in vacuum, as described by the associate referee in his report this year, be adopted as official (first action).

Approved.

(2) That during the coming year the associate referee also study methods for the detection of preservatives, coloring matters, emulsifying agents, or other added substances in cheese.

Approved.

#### MALTED MILK AND DRIED MILK.

It is recommended that the associate referee study methods for the following determinations, particularly in malted milk: (a) fat, (b) moisture, (c) cold water extract, (d) carbohydrates.

Approved.

#### ICE CREAM.

It is recommended that the associate referee continue the plans that have been formulated for collaborative studies, giving particular attention to the methods for the determination of sugars, milk solids, and gelatin.

Approved.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 260.

## FATS AND OILS.

It is recommended—

(1) That the official method for the determination of unsaponifiable residue in fats and oils<sup>1</sup> be dropped (first action).

Approved.

(2) That the F. A. C. method (see p. 45) for the determination of unsaponifiable matter, as described in this year's report of the referee, be made official (first action).

Approved.

(3) That the Kerr-Sorber method for the determination of unsaponifiable matter<sup>2</sup>, as modified by the referee and described in his report this year, be made official (first action on the modified method).

The committee does not approve this recommendation. It is believed inadvisable to have two or more methods for the same determination except under special conditions. The committee recommends that the tentative Kerr-Sorber method adopted at the 1924 meeting be dropped.

Action of committee approved.

(4) That the André-Cook method for the determination of acetyl value be studied further with reference to the time necessary for complete saponification and that castor oil or mixtures of castor with other vegetable oils be employed for this work.

Approved.

(5) That the method of Thomas and Chai Lan Yen<sup>3</sup> for the detection and determination of peanut oil alone or in the presence of other oils be investigated by collaborative study.

Approved.

## BAKING POWDERS AND BAKING CHEMICALS.

It is recommended—

(1) That the tentative electrolytic method<sup>4</sup> for the determination of lead in baking powders and baking chemicals be made official (first action).

Approved.

(2) That the tentative gasometric method<sup>5</sup> for the determination of carbon dioxide be further studied by comparing the results obtained by using a factor weight with those obtained by using a fixed weight and correcting for temperature and pressure determined at the time the volume of gas is read.

Approved.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 295.

<sup>2</sup> *This Journal*, 1924, 8: 272.

<sup>3</sup> *J. Am. Chem. Soc.*, 1923, 45: 113.

<sup>4</sup> *Methods of Analysis*, A. O. A. C., 1925, 310.

<sup>5</sup> *Ibid.*, 305.

(3) That methods be developed for the separation and determination of meta, pyro, and ortho phosphates in mixtures.

Approved.

(4) The committee further recommends that for the present studies of methods for the determination of the neutralizing value of mono-calcium phosphate be discontinued.

Approved.

#### FLUORIDES IN BAKING POWDER.

It is recommended—

(1) That the tentative method<sup>1</sup> for the determination of fluorides in baking powder and baking chemicals be modified to provide for the correction for sulfates and chlorides in the absorption solution, as described by the associate referee in his report this year (see p. 45).

Approved.

(2) That the method for the determination of fluorides in baking powder and baking chemicals as modified by Recommendation (1) be made a tentative method.

Approved.

(3) That further study of the method for the determination of fluorides in baking powder be discontinued and the associate referee on this subject dropped.

Approved.

#### EGGS AND EGG PRODUCTS.

It is recommended—

(1) That the parenthetical statement "Report results as percentages of the original sample and of the total solids contained therein" be inserted in the next revision of *Methods of Analysis* immediately under the chapter heading "Eggs and Egg Products".

The committee does not approve this recommendation for reason that necessity for same is not apparent.

Action of committee approved.

(2) That the method for sampling and preparation of sample of flaked and drum dried eggs, as described by the referee in his report this year, be adopted as tentative and combined with the tentative methods (see p. 56) for the sampling and preparation of samples of liquid and powdered dried eggs.

Approved.

(3) That the umpire vacuum method (see p. 56) for the determination of total solids in eggs, as given in the referee's report for 1924, be adopted as official (final action).

The committee does not approve this recommendation but recommends that the word "umpire" be eliminated and that the method be submitted

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 312.

to further study, including the consideration of the method of drying at 55°C. (Department of Agriculture Bulletin 846) as well as collaborative study.

Action of committee approved.

(4) That the routine air method (see p. 57) for the determination of total solids in liquid and dried eggs, as described by the referee in his report this year, be adopted as a tentative method.

Approved.

(5) That the routine air method be studied during the coming year. In this study it is suggested that each collaborator analyze a sample of fresh eggs obtained by himself and make comparison of results with those obtained by the vacuum method.

Approved.

(6) That methods for the determination of ash in eggs be studied during the coming year, particular consideration being given to the type of material of the ashing dish.

Approved.

(7) That the method<sup>1</sup> for the determination of organic and ammoniacal nitrogen in eggs, as described in the referee's report for 1924, be adopted as official (final action).

Approved.

(8) That the method<sup>2</sup> for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in eggs be studied further. It is suggested that these studies include the separation and washing of the alcohol precipitated protein free from the mother liquor by the aid of centrifugalization, as proposed in the referee's report this year, and also the complete extraction of the water-soluble proteins from the sample.

Approved.

(9) That the acid hydrolysis method<sup>3</sup> for the determination of fat in eggs, as described in the referee's report for 1924, be adopted as official (final action).

The committee does not approve this recommendation for the reason that the report of the referee indicates no collaborative study. It is recommended that the method be continued as a tentative method and subjected to collaborative study.

Action of committee approved.

(10) That the method<sup>4</sup> for the determination of lipoids and lipid phosphoric acid ( $P_2O_6$ ) in eggs, as described in the referee's report for 1924, be adopted as official (first action).

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<sup>1</sup> *This Journal*, 1925, 8: 601. See also p. 57.

<sup>2</sup> *Ibid.*, 1923, 7: 85.

<sup>3</sup> *Ibid.*, 1925, 8: 601. See also p. 58.

<sup>4</sup> *Ibid.*, 1925, 8: 602. See also p. 58.

The committee does not approve this recommendation for the reason that the report of referee does not indicate any collaborative study. It is recommended that the method be continued as a tentative method and subjected to collaborative study.

Action of committee approved.

(11) That methods for the determination of unsaponifiable matter in eggs be studied during the coming year.

Approved.

(12) That methods for the determination of acidity of lipoids in eggs be studied during the coming year.

Approved.

(13) That the study of the method for the determination of acid-soluble phosphoric acid in eggs be continued during the coming year. It is suggested that this study include consideration of (1) the addition of picric acid to the extraction mixture at the end of the half-hour period of shaking instead of at the beginning, (2) the determination of the phosphoric acid by the volumetric method, and (3) any other procedure that will simplify the method.

Approved.

(14) That the study of methods for the determination of zinc in eggs be continued during the coming year.

Approved with the further recommendation that this study be referred to the Referee on Metals in Foods.

#### FOOD PRESERVATIVES.

(1) It is recommended that the study of the sublimation method for the separation, purification, and determination of benzoic acid, salicylic acid, and saccharin when these or their derivatives are used as preservatives in food products, be continued during the coming year.

Approved.

(2) Committee B recommends that the study of the Rupp<sup>1</sup> and other methods for the determination of small quantities of chloramine products be referred to the Referee on Food Preservatives.

Approved.

#### COLORING MATTERS IN FOODS.

It is recommended—

(1) That collaborative work be undertaken on the methods for the separation of light green S F yellowish from guinea green B and yellow AB from yellow OB.

Approved.

(2) That further work be done on the methods for separating yellow AB and yellow OB from other oil-soluble dyes.

Approved.

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<sup>1</sup> U. S. Dept. Agr. Bull. No. 1114.

(3) That because of the unsatisfactory results obtained by the present tentative method, other methods for the quantitative separation of amaranth from tartrazine be studied.

Approved.

#### METALS IN FOODS.

It is recommended—

(1) That more sensitive methods for the determination of tin, copper, zinc, and aluminium be sought and that any such method found be studied collaboratively.

Approved.

(2) That the method for the determination of lead, as proposed by the referee in his report this year, be studied collaboratively.

Approved.

(3) That the work on methods for the determination of zinc in eggs and egg products, as recommended by the Referee on Eggs and Egg Products be continued under the direction of the Referee on Metals in Foods.

Approved.

#### FRUITS AND FRUIT PRODUCTS.

It is recommended—

(1) That a further comparison be made of the refractometric and official vacuum methods for the determination of solids in solutions containing sucrose and organic acids.

Approved.

(2) That consideration be given to all methods now included by reference in the chapter on "Fruits and Fruit Products" and that the referee submit to the association preliminary directions, where same are necessary, to make such methods definite and complete.

Approved.

#### FRUIT ACIDS.

It is recommended that the method for the determination of inactive malic acid, as outlined by the associate referee in his report for this year, be subjected to collaborative study.

Approved.

#### ADDED WATER IN GRAPE JUICE.

It is recommended that the tentative method<sup>1</sup> for the determination of added water in white grape juice be modified, as described in the associate referee's report for this year, and the modified method (see p. 38) subjected to collaborative study.

Approved.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 218.

## ASH IN FRUIT PRODUCTS.

It is recommended that methods best adapted for the complete analysis of the ash of fruit products be compiled with a view to including them in *Methods of Analysis*.

Approved.

## CANNED FOODS.

No report was submitted.

It is recommended—

(1) That the study of methods for the detection of spoilage in canned foods be continued.

Approved.

(2) That the recommendation of the Referee on Cereal Foods, "That the test devised by the analysts of the U. S. Bureau of Chemistry to distinguish between field and sweet corn with a view to its collaborative study and adoption as an official method", be referred to the Referee on Canned Foods.

Approved.

## VINEGARS.

It is recommended—

(1) That the method for the determination of total reducing substances before inversion<sup>1</sup> be made official (final action).

Approved. (First action taken in 1923.)

(2) That the method<sup>1</sup> for the determination of total reducing substances after inversion be made official (final action).

Approved. (First action taken in 1923.)

(3) That the tentative method for the determination of lead precipitate in vinegar<sup>2</sup> be dropped.

Approved.

(4) That the tentative method for the determination of polarization in vinegars<sup>2</sup> be dropped.

The committee does not approve this recommendation for reason that the method may be of value in the examination of sugar and malt vinegars which, apparently, was not considered by the referee.

Action of committee approved.

(5) That the methods for the determination of ash in vinegars<sup>3</sup>, particularly as they influence the subsequent determination of phosphoric acid, be studied during the coming year.

Approved.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 326.

<sup>2</sup> *Ibid.*, 329.

<sup>3</sup> *Ibid.*, 325.

(6) That the methods for the determination of phosphoric acid in vinegars<sup>1</sup> be studied during the coming year.

Approved.

(7) That further studies be made of the following methods<sup>2</sup>: (a) non-volatile reducing substances (sugar), (b) volatile reducing substances, (c) glycerol, and (d) sulfates.

Approved.

#### FLAVORS AND NON-ALCOHOLIC BEVERAGES.

It is recommended—

(1) That the Wichmann method<sup>3</sup> for the determination of the lead number of vanilla extract and imitation vanilla extracts, as described by the referee in his report for 1924, be adopted as an alternative official method (final action, first action taken in 1924).

Approved.

(2) That the chromate method<sup>4</sup> for the determination of lead, as described in the referee's report for 1924, be adopted as an alternative official method (final action, first action taken in 1924).

Approved.

(3) That the hydrochloric acid method (Method II) for the gravimetric determination of resins in vanilla extracts, as described by the referee in his report this year, be modified by the statement "Report results in grams per 100 cc. to two decimal places" and the modified method (see p. 48) adopted as tentative.

Approved.

(4) That the tentative qualitative test for vanilla resins<sup>5</sup> be modified by deleting the statement beginning "Place 50 cc. of the extract" and ending "filtrate for further tests" and changing the sentence "Place a portion of the filter with the attached resins" to "Place a portion of the dried resin".

Approved.

(5) That the qualitative test, as modified by Recommendation (4), be retained as a tentative method and the sub-heading "Qualitative Tests—Tentative" placed above same.

Approved.

(6) That final action on the Folin and Denis rapid colorimetric method<sup>6</sup>, described in the referee's report for 1924, be deferred for another year and that additional work be done with a view to determining the effect of added caramel.

Approved.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 325-326.

<sup>2</sup> *Ibid.*, 326-329.

<sup>3</sup> *J. Ind. Eng. Chem.*, 1921, 13: 414; *This Journal*, 1925, 8: 689. See also p. 47.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1914, 6: 926; *This Journal*, 1925, 8: 691. See also p. 47.

<sup>5</sup> *Methods of Analysis*, A. O. A. C., 1925, 350.

<sup>6</sup> *This Journal*, 1925, 8: 688.



(7) That the referee continue the clearing away of old unacted upon recommendations listed in the report of the referee for 1924<sup>1</sup>, and that the work begun this year on methods for the analysis of non-alcoholic flavors be continued.

Approved.

#### MEAT AND MEAT PRODUCTS.

It is recommended—

(1) That the tentative method for the determination of nitrites in meats<sup>2</sup> be studied collaboratively.

Approved.

(2) That during the coming year the referee consider the methods<sup>3</sup> for soluble and insoluble nitrogen; coagulable nitrogen; proteose, peptone, and gelatin nitrogen; amino nitrogen; meat bases; total soluble phosphorus; and separation of soluble inorganic and organic phosphorus with a view to eliminating them because they are intended only for research on the composition of meats and do not directly concern the members of the association.

Approved.

#### SEPARATION OF MEAT PROTEINS.

No report or recommendations.

#### DETERMINATION OF SUGAR.

It is recommended that studies of the method for the determination of sugar in meats be discontinued for the coming year.

Approved.

#### GELATIN.

It is recommended that the study of methods for the determination of copper and zinc be continued. This study should include collaborative work on the method described in the referee's report and the tentative methods<sup>4</sup> of the association.

#### CEREAL FOODS.

##### FLOUR.

It is recommended—

(1) That the following directions for reporting results of analyses be inserted in the next revision of *Methods of Analysis* immediately under the heading "Wheat Flour".

Report results on at least two of the following bases:

- (1) Original sample.
- (2) Total solids in sample.
- (3) 13.5 per cent moisture in sample.

<sup>1</sup> *This Journal*, 1925, 8: 687.

<sup>2</sup> *Methods of Analysis* A. O. A. C., 1925, 240.

<sup>3</sup> *Ibid.*, 243-249.

<sup>4</sup> *Ibid.*, 256.

The committee does not approve this recommendation. It seems unnecessary and might lead to confusion.

Action of committee approved.

(2) That the method for sampling flour described in the report of the associate referee be adopted as tentative (see p. 39) and submitted to further study during the coming year.

Approved.

(3) That the umpire vacuum oven method (see p. 39) for the determination of total solids and moisture (indirect method) in flour, as described in the report of the referee, be adopted as official (first action).

The committee recommends that the word "umpire" be eliminated and the method adopted as official (first action).

Action of committee approved.

(4) That the routine air-oven method (see p. 40) for the determination of total solids and moisture (indirect method) in flour, as described in the report of the referee, be adopted as official (first action).

The committee does not approve this recommendation but recommends that the method be adopted as a tentative method and that the associate referee continue the collaborative studies.

(5) That collaborative study of the glycerol method for the rapid determination of ash in flour be discontinued.

Approved.

(6) That the associate referee continue the studies on the development of rapid methods for the determination of ash in flour and that he include in these studies the use of alundum of different granulations and preferably such other procedures as yield an ash of the same composition as that produced by the official method.

Approved.

(7) That the method (see p. 40) for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour, as described in the report of the associate referee, be adopted as an official method (first action).

Approved.

(8) That the method (see p. 40) for the determination of lipoids and lipoid phosphoric acid ( $P_2O_5$ ) in flour, as described by the associate referee in his report, be adopted as official (first action).

Approved with the following changes in wording suggested by the general referee:

(a) Change the second sentence in part to read: "All the particles with the alcohol, stopper, and set in, etc."

(b) Immediately preceding the last sentence insert the phrase "Wash the residue and filter well with hot water".

(9) That the acid hydrolysis method (see p. 41) for the determination

of fat in flour, as described in the report of the associate referee, be adopted as an official method (first action).

The committee does not approve this recommendation but recommends that the method be adopted as a tentative method and further studied during the coming year. This study should include a comparison with the official method to establish the need for one or both methods; if both are desirable proper distinguishing titles should be given.

Action of committee approved.

(10) That the study of the method for the determination of glutenin in flour be continued as indicated in the report of the associate referee.

Approved.

(11) That the study of methods for the determination of hydrogen-ion concentration of flour, as outlined by the associate referee, be continued.

Approved.

(12) That the study of methods for the determination of gluten in flour be continued.

Approved.

(13) That the study of methods for the determination of the diastatic value of flour be continued.

Approved.

(14) That the method for the determination of chlorine in chlorine treated flours, as described in the report of the associate referee, be studied collaboratively.

Approved.

(15) That an associate referee be designated to study methods for the determination of starch in flour.

Approved.

(16) That methods for the determination of unsaponifiable matter in flour be studied during the coming year.

While the committee does not wish to disapprove recommendations for new work, it is felt that owing to the already large number of associate referees on cereal foods, it is unwise to assign definite lines of new work until those under way are more nearly completed.

Action of committee approved.

(17) That the tentative method<sup>1</sup> for the determination of the acidity of water extract of flour be studied with a view to the simplification of the extraction and filtration and the reporting of the results other than as lactic acid.

While the committee does not wish to disapprove recommendations for new work, it is felt that because of the already large number of associate referees on cereal products, it is unwise to assign definite lines

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 225.

of new work to be undertaken until those under way are more nearly completed.

Action of committee approved.

(18) That an associate referee be designated to study a rapid method for the determination of organic and ammoniacal nitrogen in flour with a view to securing a routine method that will yield results closely approximating those obtained by the official method.

While the committee does not wish to disapprove recommendations for new work, it is felt that because of the already large number of associate referees on cereal foods it is unwise to assign definite lines of new work to be undertaken until those under way are more nearly completed.

Action of committee approved.

#### BAKED CEREAL PRODUCTS.

It is recommended—

(1) That the sub-heading "Bread" be placed immediately under the heading "Baked Cereal Products"<sup>1</sup> in the chapter on "Cereal Foods" in the next revision of *Methods of Analysis*.

Approved.

(2) That the following directions for reporting results of analyses be inserted in the next revision of *Methods of Analysis* immediately under the heading "Bread".

Report results on at least two of the four following bases:

- (a) Original entire loaf.
- (b) Total solids in original entire loaf.
- (c) Original entire loaf of 62 per cent total solids.
- (d) Air-dried sample.

The committee does not approve this recommendation because it appears unnecessary and might lead to confusion.

Action of committee approved.

(3) That the tentative method for the determination of moisture in baked cereal products<sup>1</sup> be dropped.

Approved.

(4) That the method (see p. 42) for the preparation of sample of bread, as described by the referee in his report, be adopted as official (first action).

The committee does not approve this recommendation but recommends that the method be adopted as tentative and submitted to collaborative study.

Action of committee approved.

(5) That the method (see p. 42) for the determination of total solids

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 230.

of an entire loaf of bread, as described by the referee in his report, be adopted as official (first action).

The committee does not approve this recommendation but recommends that the method be adopted as tentative and submitted to collaborative study.

Action of committee approved.

(6) That the method (see p. 42) for the determination of total solids of the air-dried ground sample, as described in the report of the referee, be adopted as official (first action).

The committee does not approve this recommendation but recommends that the method be adopted as tentative and submitted to collaborative study.

Action of committee approved.

(7) That the routine air method for the determination of total solids in an entire loaf of bread be further studied. This study should include the use of a temperature of 130°C. as specified in the routine air oven method for total solids of flour.

Approved with the additional recommendation that the referee study also other rapid methods for this determination.

(8) That the tentative method (see p. 42) for the determination of ash in baked cereal foods be worded as given in the referee's report and adopted as official (first action).

Approved.

(9) That the tentative method (see p. 42) for the determination of protein in baked cereal products be worded as given in the referee's report and adopted as official (first action).

Approved.

(10) That further comparative studies be made of the methods for the determinations of lipoids (as directed for alimentary pastes) and of fat in bread.

Approved.

#### ALIMENTARY PASTES.

It is recommended—

(1) That the following directions for reporting results of analyses be inserted in the next revision of *Methods of Analysis* immediately under the heading "Alimentary Pastes".

Report results on at least two of the three following bases:

- (a) Original unground sample.
- (b) Prepared ground sample.
- (c) Total solids in the sample.

The committee does not approve this recommendation because it appears unnecessary and might lead to confusion.

(2) That the method (see p. 43) for taking and preparing analyst's sample of alimentary paste, described by the referee in his report, be made official (first reading) and replace the present tentative method<sup>1</sup>.

The committee does not approve this recommendation but recommends that the method be adopted as tentative, that it replace the present tentative method, and that it be submitted to collaborative study.

Action of committee approved.

(3) That the method (see p. 43) for the determination of total solids and moisture (indirect method) in alimentary pastes, described by the referee in his report, be adopted as official (first action) to replace the present tentative method<sup>1</sup> for moisture.

The committee does not approve this recommendation but recommends that the method be adopted as tentative, that it replace the present tentative method, and that it be subjected to collaborative study.

Action of committee approved.

(4) That the study of the routine air-oven drying method for the determination of total solids in alimentary pastes be continued during the coming year.

Approved.

(5) That the tentative methods<sup>2</sup> for the determinations of ash and of chlorides in ash as sodium chloride be made official (first action).

Approved.

(6) That the tentative method<sup>2</sup> for the determination of organic and ammoniacal nitrogen in alimentary pastes be made official (first action).

Approved.

(7) That the tentative method<sup>3</sup> for the extraction and identification of added color in alimentary pastes be made official (first action).

Approved.

(8) That the method (see p. 44) for the determination of protein, described in the referee's report, be made official (first action).

Approved.

(9) That the tentative acid hydrolysis method for the determination of fat in alimentary pastes<sup>2</sup> be modified to agree with the respective method for this determination in flour, as given in the report of the referee, and adopted as official (first action).

The committee does not approve this recommendation but recommends that the tentative method be submitted to collaborative study with the methods for the same determination in flour.

Action of committee approved.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 231.

<sup>2</sup> *Ibid.*, 232.

<sup>3</sup> *Ibid.*, 233.

(10) That the tentative method<sup>1</sup> for the determination of lipoids and lipid phosphoric acid ( $P_2O_5$ ) in alimentary pastes be modified to agree with the respective method for this determination in flour, as described in the report of the referee, and adopted as official (first action).

The committee does not approve this recommendation but recommends that the tentative method be studied collaboratively with the method for the same determination in flour.

(11) That methods for the determination of unsaponifiable matter in alimentary pastes be studied during the coming year.

Approved.

(12) That the tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in alimentary pastes<sup>2</sup> be modified to agree with the respective method for this determination in flour; the additional sentence "Use 50 cc. of the filtrate instead of 100 cc. with pastes containing large quantities of albumen" be added just preceding the sentence "Allow to stand overnight"; and the method, thus modified, adopted as official (first action).

The committee does not approve this recommendation but recommends that the tentative method be submitted to collaborative study with the methods for the same determination in flour.

(13) That the tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol be further modified by addition of the directions for separation and washing of the alcohol-precipitated protein by centrifugalization, as described in the report of the referee.

The committee refers this recommendation to the referee with the suggestion that the directions for the determination be given in a more simple form.

#### GENERAL.

It is recommended—

(1) That one or more associate referees be designated to study methods for the examination of whole grain, as wheat, corn, oats, barley, rye, etc. The methods should include the collection and preparation of sample and the important analytical determinations. These, where possible, should harmonize with the association methods for Cereal Foods.

The committee recommends that this be referred to the Referee on Feeding Stuffs.

Action of committee approved.

(2) That an associate referee be designated to study the test devised by the analysts of the U. S. Bureau of Chemistry to distinguish between field corn and sweet corn with a view to its collaborative study and adoption as an association method.

<sup>1</sup> *Methods of Analysis*, A. O. A. C. 1925, 233.

<sup>2</sup> *Ibid.*, 232.

The committee recommends that this be referred to the Referee on Canned Foods.

Action of committee approved.

#### CACAO PRODUCTS.

It is recommended—

(1) That the Lepper-Waterman method for the determination of fat in cacao products<sup>1</sup> be adopted as an official method (first action).

Approved.

(2) That the modified method for the determination of fat in cacao products, described in the report of the referee, be further studied.

Approved.

(3) That further studies be made of the methods<sup>2</sup> for the determination of casein, sucrose, and lactose in cacao products.

Approved.

#### MICROSCOPICAL METHODS.

It is recommended that the study of methods for the estimation of shell in cacao products be continued.

Approved.

#### CRUDE FIBER.

It is recommended that the method for the determination of crude fiber be studied during the coming year. This study should include the use of filter paper as compared with the procedure of the general official method<sup>3</sup> for filtering and the crude fiber content of alkali treated cacao products.

Approved.

#### CACAO BUTTER.

It is recommended that the study of methods<sup>4</sup> for the detection of foreign fats in cacao butter be continued.

Approved.

### THIRD DAY.

## WEDNESDAY—AFTERNOON SESSION.

### REPORT OF COMMITTEE TO COOPERATE IN REVISION OF THE UNITED STATES PHARMACOPEIA.

The United States Pharmacopeia, 10th decennial revision, came from the press last August, and it becomes official January 1, 1926. This committee desires to call attention to several features.

The terms "official" and "pharmacopeia" are considered synonymous. The revision contains 100 fewer pages than the 9th edition. Forty medicaments have been added and 191 deleted. The number of drugs

<sup>1</sup> *This Journal*, 1925, 8: 706. See also p. 46.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 343-4.

<sup>3</sup> *Ibid.*, 118.



provided with pharmacopeial standards is therefore considerably reduced. The difference between the number of products added and those deleted accounts in a measure for the decrease in the size of the publication, but other features also help to reduce the size. For example, numerous repetitions heretofore scattered throughout the body of the text are now condensed and brought under appropriate headings.

The reduction in the number of drugs included in the Pharmacopeia is in conformity with the trend of medical practice. Advancement in medical art is also reflected by the nature of the additions, among which may be mentioned the following: Ethyl chaulmoograte, for treating leprosy; arsphenamine, used for syphilis; carbon tetrachloride, for eliminating intestinal parasites, hook worm in particular; chloramine, a useful germicide; procaine hydrochloride, to replace cocaine in part; and epinephrine and thyroxin, representing advancement in endocrine therapy.

Among the products deleted are a goodly number of alcoholic preparations. For example, twenty-four fluid extracts are deleted and two added; four spirits are dropped and two added, the latter being whisky and brandy; and fifteen tinctures have been eliminated but no new ones added. It will be noted that tests for the possible presence of denatured alcohol are included in the monographs on whisky and brandy.

A statement appears as to the range of permissible alcohol content in conjunction with each alcoholic preparation; it varies from 3 to 11 per cent, depending upon concentration of the alcohol.

In the manufacture of resins, extracts, etc., in which alcohol is used as a solvent but removed from the finished product, it is permissible to use non-potable alcohol containing from 5 to 10 per cent by volume of commercially pure methanol or acetone.

The standards of purity and strength prescribed for the various products are intended to apply solely to substances that are used for medicinal purposes, and professedly when bought, sold, or dispensed as such.

Atomic weights are those adopted by the International Committee on Chemical Elements (1921) Oxygen = 16.

The tenth decennial revision contains an excellent history of the development of the Pharmacopeia, prepared by E. Fullerton Cook, Chairman of the Committee of Revision.

The term mil has been replaced by the abbreviation cc. Twenty-five degrees centigrade is the standard temperature for determining solubilities, specific gravities, polarimetric readings, making volumetric solutions, etc. In the case of alcohol the 60°F. (15.56°C.) has been retained on account of the legal requirements and regulations of the Internal Revenue Bureau of the United States. "For refractive indices, viscosity, and for certain saccharimeters temperatures other than 25°C. have been directed as the special conditions require".

With the exception of pituitary solution, cannabis and its preparations, biological tests were optional in the ninth decennial revision, but they are now obligatory for a number of important drugs. The chemical assay for aconite has been omitted, but the biological assay is retained. An optional assay method for determining Vitamin A in cod liver oil has been introduced. This is in harmony with the advancement of vitamin studies.

Heroin has been omitted, which conforms with Federal legislation controlling narcotics.

The unsatisfactory U. S. P. IX instructions for determining the quantity of distillate, in case of cresol between 195° and 205°C., have been replaced by a definite course of procedure. This committee had an active part in bringing about this change. Whether or not the new procedure is wholly satisfactory will remain for the future to determine.

The next problem will be to try out the new standards and thus find out how well the work has been done.

With the appearance of the Pharmacopeia, the duties of this committee cease. Its members, however, desire to express to the Committee of Revision, through the association, its great appreciation of the privilege accorded in taking part in the revision of the Pharmacopeia, and it is recommended that a communication to this effect be transmitted to the Chairman of the Committee of Revision.

L. F. KEBLER,	J. M. DORAN,
H. C. LYTHGOE,	H. C. FULLER.
A. R. BLISS, JR.,	

*Committee to Cooperate in Revision of the  
U. S. Pharmacopeia.*

Approved.

## REPORT OF THE REPRESENTATIVES OF THE A. O. A. C. ON THE BOARD OF GOVERNORS OF THE CROP PRO- TECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL.<sup>1</sup>

Time will be taken for only a brief accounting of the work of the institute not heretofore reported and for making reference to publications that have appeared under its auspices.

The publication of the results of the cooperative dusting and spraying experiments on the control of diseases and insect pests of apples and peaches was completed in the Crop Protection Digest, No. 4, the former report having appeared in No. 2.

The results have been reported of the third year, 1924, of cereal seed

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<sup>1</sup> Presented by B. E. Gilbert.

treatments at experiment stations in the United States and Canada. They involve especially treatments of bunt and smut with copper carbonate, formaldehyde, nickel carbonate, and chlorophenol mercury.

The sulfur investigations supported for two and a half years jointly by the Texas Gulf Sulphur Company, the Freeport Sulphur Company, and the Union Sulphur Company were closed in the fall of 1924. The following papers on the sulfur investigations have been published in Nos. 3, 5, and 6, respectively, of the Crop Protection Digest:

The Toxic Properties of Sulphur, by Harry C. Young.

An Investigation of Sulphur as an Insecticide, by Albert Hartzell and F. H. Lathrop.  
Spray Injury to Apples, by Harry C. Young and R. C. Walton (published also in Phytopathology, Vol. XV, No. 7).

Investigation of crown gall for two years was made possible by contribution from the Universities of Iowa and Wisconsin, and the American Association of Nurserymen. "Reports of Progress on Studies of Crown Gall in Relation to Nursery Stock" have been made by A. J. Riker and G. W. Keitt in "Science", N. S. 62, 184-185, 1925.

In December, 1924, a two-year contract was made with the Standard Oil Company of Indiana for the investigation of a new type of emulsified oil.

In July, 1925, a two-year arrangement was made with the Standard Oil Company of New Jersey for studying the insecticidal value of their product called "Flit".

A fundamental study for two years of the use of various copper salts in agriculture has been arranged through the cooperation of several companies.

BURT L. HARTWELL,  
H. J. PATTERSON.

Approved.

## REPORT OF THE SECRETARY-TREASURER.

By W. W. SKINNER (Bureau of Chemistry, Washington, D. C.).

Further improvement can be recorded this year in every branch of the association's work. The balance in the bank is \$898.96 compared with \$685.54 last year.

An unusually large number of changes have occurred in referees and associate referees since the publication of the list. W. J. Clarke was appointed Referee on Metals in Foods in place of B. J. Hartmann, resigned; A. C. Dahlberg was appointed Associate Referee on Ice Cream in place of Jacob Moyer, resigned; E. W. Schwartze was appointed Referee on Bio-assay of Drugs; E. B. Kress was appointed Associate Referee on Gluten in Flour; and C. O. Swanson was appointed Associate

Referee on Starch and Diastatic Value of Flour. No appointment was made to fill the place of A. L. Sullivan, Referee on Canned Foods, and no appointment was made of an Associate Referee on Ether. William Seaman, the Associate Referee on Honey, resigned, and no appointment has been made, although considerable correspondence was carried on with the directors of the Experiment Stations in the States producing the largest quantities of honey.

One vacancy in the Committee on Definitions of Terms and Interpretation of Results on Fertilizers has occurred owing to the death of Edward George Proulx. The secretary regrets that it is necessary to make this record as well as to announce the deaths of Senator E. F. Ladd and C. L. Penny, past presidents of the association, Guilford L. Spencer, and A. L. Burns. All these men had been active in the association's affairs. An obituary notice for Mr. Proulx was published in Volume VIII, No. 5, and one for Senator Ladd will be published. (See page iii.) A testimonial of Dr. Spencer's contributions as a sugar technologist in the Bureau of Chemistry and as referee for this association was written by Dr. C. A. Browne and published in "The Reference Book of the Sugar Industry of the World", July, 1925.

Many letters addressed to the secretary or to the Bureau of Chemistry relating to special work have been referred to the proper referees, and some of the points have been discussed by them in their reports.

The secretary was also one of a committee of two to devise, if necessary, ways to finance the 1925 edition of *Methods of Analysis*, but it was not necessary to formulate any new plans. The sale of the book continues to be good, as the detailed report of the Chairman of the Board of Editors has shown.

At a meeting of the Executive Committee last evening the following matters were decided:

(1) That the reports of referees, where practicable, should be presented in abstract, in order to encourage debate.

(2) That a summarized report of each meeting should be prepared by the Committee on Editing Methods of Analysis. This report should present in concise form the changes in the methods adopted at the meeting and be published in the first issue of *The Journal* after said meeting.

(3) That, as recommended by the Committee on Editing Methods of Analysis, it should be the policy of the association, so far as practicable, to have but one accepted official method for each determination.

There is now presented for consideration the following very important communication from Dr. H. W. Wiley:

## GOOD HOUSEKEEPING.

Bureau of Foods, Sanitation and Health.

Harvey W. Wiley, M. D.  
Director.

506 Mills Building,  
Washington, D. C.

June 13, 1925.

Dr. W. W. Skinner, Sec'y,  
Association of Official Agricultural Chemists,  
Box 290 Pa. Ave. Station,  
Washington, D. C.

Dear Doctor Skinner:

I have just finished the third revision of Volume I of "Principles and Practice of Agricultural Analysis". There is an immediate need for the third revision of Volume II and III of this book. Due to my failing eye sight and my being out of contact with the progress in agricultural chemistry, I have decided to present this work to the Association of Official Agricultural Chemists.

I have already spoken to some of the members in regard to this matter, and I hope the offer which I make will be acceptable. At the present time the copyright of my book, "Principles and Practice of Agricultural Analysis" rests in me. I purpose to present to the Association of Official Agricultural Chemists the copyright of the second and third volumes of the third revision, and the copyright of all the volumes of the fourth and all subsequent revisions, to have and to hold in the interest of agricultural science perpetually.

The royalties from Volumes II and III, third revision, are to be divided as follows: 10 per cent of the total royalty to revert to me, as my final proprietary interest in the book; for the fourth edition of all the volumes, all the royalties will be the property of the Association of Official Agricultural Chemists. The royalties from this book are far larger than had been anticipated. For twenty years or more they have amounted to several hundred dollars per year. These royalties can be used by the association as it sees fit. I think the greater part of the royalty should accrue to those who undertake the subsequent revisions in proportion to the amount of labor done by each one. A small percentage, say 15 or 20 per cent, of the total proceeds I should like to be devoted to the formation of a permanent fund, which would increase from year to year in proportion as the books were sold, and the interest from this fund to be used by the association in promoting the improvement of these volumes, and the principal held in trust for this purpose.

It is my desire that Dr. Charles A. Browne, Chief of the Bureau of Chemistry, should be the chairman of the committee on these revisions, and that the association consult him in regard to the employment of various workers in the different fields.

It is my desire also that the character of the work shall remain as it is at the present time, with the same name, and with my name on the title page in perpetuity, but the property right or copyright of the book shall be permanently in the name and for the service of the Association of Official Agricultural Chemists, in keeping the book up to the latest development of progress in analytical chemistry relating to agriculture.

I would like that particular stress be laid on the principles of analysis rather than on the details.

It is also my wish that the plan which I have long cherished should be carried out; namely, to prepare a laboratory manual for workers in laboratories, from this book, in which not only the official methods of the association are used, but also standard methods of other countries, such as England, Germany, France, and Italy. This manual should also bear my name on the title page, and the name of the present work.

These are the general principles which I would like to see carried out in the life of this work. I believe that the Association of Official Agricultural Chemists is the most competent body existing to keep this work up to the highest state of efficiency. There will be, of course, a number of details to be worked out with the officials of the organization which are not touched by this general outline, but if the association will accept this work substantially as indicated in this Deed of Trust, I am sure all the minor details can be easily arranged.

I beg you to bring this matter to the attention of the executive committee as soon as possible, in order that a definite plan can be agreed upon by the time the association holds its next annual meeting, which I presume will be in Washington next fall.

With sentiments of highest consideration, I am,

Very sincerely yours,

(Signed) H. W. WILEY.

The matter was discussed at the summer meeting of the Executive Committee and the secretary sent letters to past officers of the association and other members in order to get an expression of opinion as to the practicability of taking over this offer of Dr. Wiley's, which sentiment at first seemed to dictate should be accepted. Dr. Wiley, however, in his letter named some conditions, or what it was thought were conditions, which seemed to make it doubtful if they could be met by the association. The laboratory manual which he mentioned, the committee concluded, was impracticable. The other points about which there seemed to be some doubt in the mind of the committee have been cleared up in subsequent conferences of Dr. Wiley, Dr. Browne, Dr. Doolittle, and the secretary, so that the seeming impossible conditions which Dr. Wiley put in his first letter may now be regarded as mere suggestions, and he is entirely willing to turn over the copy of the volumes to the association practically without restrictions. The difficulty of obligating the association to carry on the work of revision at some future time was explained to him. He agreed that if at any time the association did not wish to go on with this work it might be turned back to his heirs. The royalty from the three books, Dr. Wiley told the committee, amounts to from \$400 to \$500 a year. He said that he had received in royalties several thousand dollars. The book had had a greater demand than he had anticipated and a very definite demand from abroad, especially England. It would seem, therefore, that based upon previous demand a revision might be expected to yield approximately \$5,000 in the course of 10 years.

The committee has thought—and Dr. Wiley has agreed to it—that the book might be revised by chapters, various chapters being assigned to persons who are familiar with the work in this association and most competent to handle it and each person to be paid for his services pro rata out of the royalties received. In addition, the name of the author of the revised chapter should appear in the title of that particular chapter. If the gift is accepted, the plan, of course, will necessitate the

appointment of an editor-in-chief. Dr. Wiley has suggested his preference for Dr. Browne. That is very natural, of course, because of his close association with Dr. Wiley and because of his eminent fitness for the position. The plan also provides for the appointment of a board which should have, at least as some of its members, the present editorial committee of the association, because Dr. Wiley's book should be a supplement to and harmonize with *Methods of Analysis*.

The financial statement will be given and perhaps after that further discussion of this matter may be had. (For financial statement see p. 64).

Approved.

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*C. A. Browne:* In regard to the matter which Dr. Skinner brought up, regarding Dr. Wiley's donating the copyrights of his book, I might say that I have had several conferences with Dr. Wiley, and as Dr. Skinner indicated, Dr. Wiley is willing to have the association arrange for the publication as it wishes, the only condition being that his name appear on the title page. The principal incentive for our undertaking the revision would be to make the book useful to the association, and this might be possible if we had it appear as supplementary to the chapters of our own book. I think it could only be on that plan that the association would be justified in accepting Dr. Wiley's offer.

There is room, however, for discussion of this matter, and the Executive Committee would like to hear from some of the members present if they care to take the matter up at this time.

*B. B. Ross:* Mr. President, I was very much pleased to hear of this very generous offer of Dr. Wiley's. It showed, on his part, a very marked confidence in the association and appreciation of our work. At the time that Dr. Skinner mentioned this, I recognized the difficulties lying in the way, especially along the lines laid down by Dr. Wiley. But now that the stipulations have been either withdrawn or modified so that the plan would be practical, I should like very much to see it carried out. I think we should adopt a resolution of appreciation of Dr. Wiley's generosity and of his confidence.

*C. A. Browne:* Do you make that a motion?

*B. B. Ross:* I do.

Motion was seconded and unanimously passed.

*C. A. Browne:* Is there any other discussion in regard to this matter? I had a little talk in my office a few months ago with the publisher and I asked him about what the royalties were, at the present time, from each one of these volumes. He indicated to me that it was about \$200 and that that was what we could expect. This sum would pay the contributors in the course of 8 or 10 years.

Is it the opinion of the association at this time, after hearing the presentation of Dr. Skinner and what Dr. Ross has said, that we are justified in accepting Dr. Wiley's offer? If not, the matter could be referred to a committee.

*G. S. Fraps:* Mr. Chairman, I move that the offer of Dr. Wiley be accepted and that the matter be referred to the Executive Committee with power to act.

The motion was seconded.

*W. W. Randall:* Wouldn't that acceptance be provisional upon the committee being able to secure such cooperation as would enable it to go ahead?

*C. A. Browne:* I think that might be included if there is no objection. Then, all in favor of accepting Dr. Wiley's offer will signify by saying "Aye!"

Unanimously approved.

## REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS.

This committee respectfully submits the following report covering the proceedings of the Joint Committee on Food and Drug Definitions and Standards during the past year:

Two meetings of the committee were held, one from February 24th to March 4th, and another during the week beginning July 13th.

### FEBRUARY-MARCH MEETING.

The February-March meeting, which was of unusual length, enabled the committee to hold several hearings and to devote adequate attention to a number of important subjects. A conference was held with individuals representing the principal manufacturers of almond paste and kernel paste. Much important first-hand information was obtained regarding the character, composition, and methods of manufacture of these products, and after some discussion on the part of the committee tentative definitions and standards were adopted to be submitted to the trade for criticism.

The committee held an interesting and profitable conference with manufacturers of sauerkraut, and in the discussion of this subject valuable assistance was received from E. Lefevre of the Bureau of Chemistry Microbiological Laboratory. The manufacturers were concerned chiefly with the question of formulating a definition that would adequately define their product and serve to protect the industry from a variety of loose practices, notably the production and sale of immature kraut and products bordering on ordinary salted cabbage or slaw. After con-



siderable discussion tentative definitions and standards were adopted for sauerkraut, in bulk and in can.

Considerable time was given to a further discussion of the present definitions for alimentary pastes as they now appear in Circular 136. A number of vital questions have arisen recently in connection with these products. A lively conference was held with B. R. Jacobs, Executive Secretary of the American Macaroni Manufacturers Association, relative chiefly to the grades of flour and semolina used in the manufacture of macaroni and the standard for egg solids in egg pastes. After a prolonged discussion of these subjects the committee adopted a tentative schedule including, first, a generic definition for alimentary pastes; second, a definition for plain pastes (macaroni, spaghetti, vermicelli, water noodles, etc.); and, third, a definition and standard for egg pastes (noodles, egg noodles, egg vermicelli, etc.). A moisture standard of 13 per cent, applicable to all alimentary pastes, was adopted tentatively, and a change was made to a 5.5 per cent egg solids standard for egg pastes, expressed on a moisture-free basis. Further consideration of this subject was postponed until a later meeting.

Much time was given to a further consideration of the fruit products schedule, and valuable assistance was rendered by a number of representatives from the Bureau of Chemistry and by Louise Stanley, Chief of the Bureau of Home Economics. Hearings were given to representatives of the National Preservers Association and also, on a separate day, to representatives of the Douglas Packing Company. The fruit products schedule has been much delayed and rendered increasingly difficult on account of the commercial and domestic use of pectin preparations in the making of jams, preserves, jellies, and marmalades. After a prolonged discussion of this schedule the committee approved tentative definitions and standards for the following terms: fruit; fresh fruit; dried fruit (a) "sun dried", (b) "evaporated", (c) "dehydrated"; cold-pack fruit; canned fruit; preserve, fruit preserve; dextrose preserve and glucose preserve, or corn sirup preserve; jam, fruit jam; dextrose jam and glucose jam, or corn sirup jam; fruit butter; dextrose fruit butter and glucose fruit butter, or corn sirup fruit butter; jelly, fruit jelly; dextrose fruit jelly and glucose fruit jelly, or corn sirup fruit jelly; and citrus fruit marmalade.

An important conference was held with H. J. Ayers, representing the Horton Ice Cream Co., New York City, with reference, chiefly, to the subject of overrun in the manufacture of ice cream. The committee was enabled thereby to obtain much valuable first-hand information from an expert of many years' experience. The hearing also covered important subjects such as variations in weight and capacity of cans, composition of filled cans, variations in overrun, composition of varieties of ice cream mix, standards for milk fat, milk solids, etc. No formal action was taken

regarding the standard that was adopted tentatively during the previous year. This ice cream subject has been a difficult one to handle owing, chiefly, to the wide diversity of standards now in effect in the various states and also to the radically different views held by individuals in different localities, including consumers as well as manufacturers.

The committee agreed to a change in the definition for butter fat as now given in Circular 136, by deleting the numerical limits.

#### JULY MEETING.

The second meeting, held in July, was given over largely to a continued discussion of the schedules that were adopted in tentative form during the meeting held earlier in the year. After a consideration of criticisms submitted by manufacturers some minor amendments were made in the definitions for various alimentary pastes, but final adoption was postponed for the reason that the manufacturers' association at a recent meeting had appointed a committee to consider the standards. It was deemed unwise to take final action until a report was received from that committee and a further opportunity given for discussion of the definitions proposed. After a brief formal conference, the committee finally approved and adopted definitions for almond paste and kernel paste, as follows:

*Almond Paste* is the plastic product obtained by cooking blanched and ground sweet almonds with blanched and ground bitter almonds, sugar, and water. It contains not more than fourteen per cent (14%) of water nor more than forty per cent (40%) of total sugars expressed as invert sugar.

*Kernel Pastes* are the plastic products obtained by cooking, with sugar and water, the blanched and ground kernels of one or more of the following: apricots, peaches, plums (prunes). They are free from hydrocyanic acid and contain not more than fourteen per cent (14%) of water, nor more than forty per cent (40%) of total sugars expressed as invert sugar. A kernel paste conforms in name to the kind or kinds of kernels employed in its production.

Following the customary procedure, these definitions and standards have been approved by the Department of Agriculture and promulgated in Food Inspection Decision 197, issued under date of August 27, 1925. The proposed tentative definition and standard for sauerkraut was taken up for discussion, and as a result the committee agreed to the adoption of the following:

*Sauerkraut* is the clean, sound product, of characteristic acid flavor, obtained by the full fermentation, chiefly lactic, of properly prepared and shredded cabbage in the presence of not less than two per cent (2%) nor more than three per cent (3%) of salt.

It contains, upon completion of the fermentation, not less than one and one-half per cent (1.5%) of acid, expressed as lactic acid. Sauerkraut which has been rebrined in the process of canning or repacking contains not less than one per cent (1%) of acid, expressed as lactic acid.

This definition and standard has also in due course been promulgated by the Department of Agriculture in Food Inspection Decision 196.

The greater portion of a day was devoted to an extended hearing on the proposed schedule of definitions and standards for fruit products. The conference was largely attended by individuals representing manufacturing firms, associations, and various departments of the Government. Much time was again devoted to a lively discussion of the use of pectin in the manufacture not only of genuine articles but also of products to be known as imitations. The proposed standard for soluble solids in jellies, jams, and preserves was given due consideration and also the subject relating to the proportion of fruit to sugar.

On a later day in the week a conference was held with R. W. Williams, Solicitor of the Department of Agriculture, and P. D. Cronin, Assistant Attorney in the office of the solicitor, relative chiefly to the legal aspects involved in the use of dried fruit in the preparation of jelly and fruit butter. The opinion of the solicitor was obtained also relative to the legal interpretation of the term "buttermilk" as applied to a class of well-known fermented milk beverages.

The committee held a conference and a prolonged discussion with members of the Bureau of Chemistry on the subject of a proposed change in the moisture standard for wheat flour. As doubtless well understood, the change has been suggested owing to certain developments that have recently taken place in connection with the adoption of vacuum drying methods for the determination of moisture. Extensive valuable data were submitted to the committee, and useful information was obtained but no formal action was taken. A conference was held with a representative of a Chicago manufacturer during which a number of questions were raised relative to the correct interpretation of the present definition of malted milk. The subjects under special consideration involved the character of the ingredients and the methods of combination; also there were some questions raised regarding the standard for fat. After prolonged discussion the committee voted that this subject be taken up for investigation during the ensuing year, with a view to the formulation of a new definition and standard for this product.

Sweet cream butter was the subject of a conference at which were present C. W. Larson, Chief, Bureau of Dairying, Department of Agriculture. The discussion related principally to the tentative definition for this product, which was first presented to the Committee for discussion during the meeting held in August, 1924. The information obtained tended to justify a definition of this product as distinct from other types of butter, and no reason developed that called for a modification of the tentative definition. As a result of some discussion, however, no final action was taken, and the subject was continued for further consideration at a future meeting. The committee concluded the session with a

formal discussion of the term "buttermilk". Because of the wide diversity of views expressed by a number of individuals, it seemed difficult to formulate a satisfactory distinction between the well-known article that results as a by-product of churning cream and the other class of products resulting from the treatment of milk or skimmed milk with culture media.

JULIUS HORTVET,                      E. M. BAILEY.  
C. D. HOWARD,  
*Committee to Cooperate with Other Com-  
mittees on Food Definitions.*

### REPORT OF COMMITTEE ON SAMPLING.

The Committee on Sampling presents the following report of progress:

(1) The individual members, chosen by reason of their familiarity with the various fields of activity of this association, have agreed during the coming year to prepare a complete bibliography on experimental work and suggestions for the proper sampling of the various types of products.

(2) The committee, after consideration of the above secured data, will prepare a suggested outline of work for consideration by the various referees for the year 1927.

The committee therefore recommends that it be continued for another year, with the idea of turning over the actual development of sampling procedures to the various referees at the earliest possible moment.

F. C. BLANCK,                      R. W. FREY,  
C. C. McDONNELL,                A. G. MCCALL,  
ARTHUR E. PAUL,                F. W. ZERBAN,  
R. N. BRACKETT,                J. W. SALE,  
A. J. PATTEN,                      J. W. KELLOGG.  
*Committee on Sampling.*

### REPORT OF COMMITTEE TO CONSIDER THE ADVISABILITY OF STUDYING METHODS FOR THE ANALYSIS OF PAINT<sup>1</sup>.

On recommendation of the Executive Committee, the association, at its meeting in 1922, authorized the appointment of a special committee to make a preliminary investigation of the methods of analysis of paints and of paint materials with the view of submitting recommendations with regard to the development, through collaborative study in the usual way, of official methods for the examination of these products.

<sup>1</sup> This report, although sent by the chairman of the committee by special delivery letter in ample time to have been received by the secretary for presentation at the meeting, failed to reach him until after the meeting.

The committee was appointed, and a general survey was undertaken of the status of paint and oil legislation in the various States and of the methods of analysis required in the enforcement of the laws and regulations found to exist. As a result of these preliminary investigations, this committee was impressed with the fact that paint, oil, and varnish analysis and control present several aspects that appear to require close examination on the part of the association.

In the first place, the need of paint and oil inspection and control has not been recognized as definitely and generally as that for the inspection and control of fertilizers, feeding stuffs, and foods. As a result, a comparatively small proportion of the membership of the association is immediately concerned with these problems.

Then, too, a casual reading of the State laws now in effect is sufficient to show the wide diversity that exists in legislative requirements and to establish a distinct need for a comprehensive consideration of the entire subject of paint and oil legislation. Laws of one kind or another already exist, and the enforcing officers are in urgent need of a better program of procedure than can now be found.

There is a close analogy in this connection with the history of the development of fertilizer, feed, and food legislation, and paint and oil law administrators and inspectors are certain to find themselves following paths that are not new to the members of this association.

Because the chemical and physical examinations to be made will depend upon statutory requirements, it is first essential to determine what these requirements shall be, and, here again, the technical work in which this association is engaged may not be studied apart from such primary considerations as the development of adequate laws, regulations, definitions, standards, etc. There must be accomplished finally a uniformity of view and of procedure that will enable each State to utilize effectively the results of the collective study and judgment of specialists in paint and oil legislation and in methods for the analysis of these products.

And there is another pertinent consideration that may not be overlooked. When this association undertook wholly similar problems in the fields of fertilizer, feeds, and foods, there was available a vast background of fundamental research, which formed a base upon which to construct. Relationships and functions were more clearly set out than one can yet find to be the case in studying paints and paint materials. The relative values of various products and of mixtures and of methods for their examination seem less clearly defined. One may be assured that much time and labor remain to be spent before the scientific control of paints will present as satisfactory a condition as this association is now enabled to contemplate in its more familiar fields of endeavor.

The association has asked this special committee for a definite recommendation regarding the desirability of making collaborative studies of

methods of paint and oil analysis, and its patience has perhaps been taxed by a recital of conditions that cause the committee to conclude that the question of this policy may not be put within limits as narrow as that. It is rather for the association to decide whether it shall co-operate with the officials of experiment stations, food and drug departments, State chemists, and State departments of agriculture in the development and in the unification of legislation and regulation, as well as in the collaborative study of methods of examination best adapted for the prosecution of effective regulatory work. Perhaps some will consider a separate organization necessary for a part of this work. In any event, the beginning can be most advantageously made here. The problem must be studied as a whole, even though the association may consider that it is concerned with only a part of it.

Most of the members of this association know that the American Society for Testing Materials has already accomplished much work upon specifications and methods for testing paint materials. The work of that society has dealt largely so far with the raw materials.

Last year this committee recommended a further investigation of the desirability of collaborative work on methods of analysis and of a study of paint legislation and suggested the calling of a convocation of administrative officers and chemists for the consideration of these questions.

The committee has now had an opportunity to consider more fully the various phases of the problem assigned to it, and the conviction prevails that much work must be done before proper aid can be given to the members of the association who are engaged in paint and oil control work. But the committee believes that there is an opportunity here for a distinct service that should not be overlooked.

The committee is also of the opinion that the association should take part in the development of paint and oil control work as it has done so successfully in other divisions of regulatory activities.

It should be pointed out that officials in seventeen States are already engaged in paint and oil inspection. There are departments also for the inspection of oils and turpentine in seven others, making twenty-four States in which paint and oil legislation exists.

The laws in the several States can not be enforced without methods for the evaluation of the product inspected. The officers in charge of the inspection are almost without exception dairy and food commissioners, commissioners of regulatory divisions of experiment stations. The inspectional work has not yet attained large proportions. It is undoubtedly hindered by a lack of definiteness and of coordination and of clear, definite, and adaptable methods of analysis, such as have been developed in this organization for other lines of work.

In view of the facts and circumstances that have been outlined, this committee recommends<sup>1</sup>:

(1) The appointment of a permanent committee of five members interested in paint and oil control work.

(2) That this permanent paint committee be instructed to begin co-operative work with the State departments engaged in paint law enforcement with the view of developing a suitable law or laws; of bringing about the uniformity of laws; and of developing and unifying regulations, definitions, and standards where necessary.

(3) That the committee be asked to cooperate in the development of methods of paint examination and analysis; to seek cooperation of paint chemists within this association's membership and elsewhere; and to cooperate with the work of the committee on Protective Coatings of the American Society for Testing Materials with the view of developing suitable procedures for the evaluation of paints, oils, and paint products.

W. F. HAND,

W. T. PEARCE.

J. W. KELLOGG,

*Committee to Consider the Advisability of Studying  
Methods for the Analysis of Paint.*

## REPORT OF COMMITTEE ON BIBLIOGRAPHY.

It may be recalled that last year a report was made by the committee regarding the preparation of a review and a bibliography of the work done by this association and by others in the same line of endeavor and that the association instructed the committee to put in operation the plan presented at the last meeting. The plan is for a review—a critical review—of the literature with a bibliography attached, arranged for each chapter of *Methods of Analysis*. After consulting with some of the officers of the association, it was decided to start the plan by selecting six important chapters of the book and appointing a person to undertake the preparation of each review. The chapters selected—not exactly chapters but subsections, as the chapter of fertilizers was divided into three parts—were potash, phosphoric acid, nitrogen, feeding stuffs, soils, and insecticides and fungicides. For a review of the work on potash, B. B. Ross, of Alabama, was selected; phosphoric acid, R. N. Brackett, of South Carolina; nitrogen, H. B. McDonnell, of Maryland; feeding stuffs, H. H. Fuller, of Texas; the chapter on soils, W. H. MacIntire, of Tennessee; and the chapter on insecticides and fungicides, C. C. McDonnell, of the Bureau of Chemistry. All of these gentlemen have accepted, and the plan will go forward this year. They were requested to prepare

<sup>1</sup> No action on these recommendations was taken at this meeting for the reason stated in the footnote on p. 107.

the review, if possible, and have it ready for presentation to the association at the meeting in 1926. As you may also remember, it is proposed to bring the review up to the date of the present edition of *Methods of Analysis* and then to have these reviews published every five years coincident with the issuing of a new edition. This review and bibliographical work will fit in very happily with the plan, if it materializes, for the association to take over the revision and publication of Dr. Wiley's "Principles and Practice of Agricultural Analysis".

W. W. SKINNER,	H. D. HASKINS,
G. S. FRAPS,	W. W. RANDALL.
F. P. VEITCH,	

*Committee on Bibliography.*

Approved.

### REPORT OF AUDITING COMMITTEE.

The Auditing Committee has examined the accounts of R. W. Balcom, Chairman of the Board of Editors, covering the period from October 1, 1924, to October 15, 1925, and found the same to be correct as reported.

The committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from October 1, 1924, to October 15, 1925, and found the same to be correct as reported.

H. B. McDONNELL,
J. B. WEEMS.

*Auditing Committee.*

Approved.

### REPORT OF NOMINATING COMMITTEE.

The committee congratulated itself prematurely on the pleasure and pride it would have in nominating H. D. Haskins, of Massachusetts, for president. Unfortunately, it learned that there are reasons that can not be ignored that prevent Haskins from serving. The committee desired unanimously to nominate Haskins, whether or no, but it has had to defer to his wishes in this matter.

The committee, therefore, desires to present the following names:

*President:* W. W. Randall, Baltimore, Md.

*Vice-President:* W. H. MacIntire, Knoxville, Tenn.

*Secretary-Treasurer:* W. W. Skinner, Washington, D. C.

*Additional members of the Executive Committee:* Oswald Schreiner, Washington, D. C., and E. M. Bailey, New Haven, Conn.

F. P. VEITCH,	H. H. HANSON.
A. G. MCCALL,	

*Nominating Committee.*



It was moved, seconded, and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

*W. W. Randall:* I have noticed, gentlemen, that there doesn't seem to be any tendency on the part of this building to fall. The feeling of your nominee is, therefore, not shared by the foundations of this structure. I can not imagine any particularly good reason for the choice which the committee has made, unless it is the rather comforting one that my first two initials correspond with those of our most excellent secretary. If it be your pleasure that I make an effort, however feeble and unsatisfactory, to do the important work which you have asked me to perform, I shall certainly undertake it with thanks for your consideration and with real appreciation of the high and most undeserved honor with which you have associated my name. Please, therefore, forgive me, for this has come with such surprise that I have not ready—even if I could ever have ready—a proper response to make to this most delightful, but still, from the standpoint of my capacity, burdensome honor. I naturally beg you not to compel me to give my honest opinion with regard to the suitability of your choice!

Let me, therefore, once more express to you my most sincere thanks.

## REPORT OF COMMITTEE ON RESOLUTIONS.

### GENTLEMEN OF THE ASSOCIATION:

The year which has elapsed since the last meeting of this association has taken heavy toll of its membership. Of the six men whose deaths must be here recorded, hardly one had reached the age when he would be willing to look upon his productive period as clearly passed. Most pathetic of all is the passing of young men in full possession of all their natural gifts.

In the case of two of those about to be mentioned, extended notices have already appeared. Others will be appropriately brought to your attention in the near future.

The roll is as follows:

Albert L. Burns,  
Edwin F. Ladd,  
Charles L. Penny,  
Edward George Proulx,  
Guilford L. Spencer, and  
Charles Dayton Woods.

Albert L. Burns, in charge of the New Orleans station of the Bureau of Chemistry for nearly two years, died in July last, leaving a widow and five children.

Born in Indiana, he was graduated from Wabash College; pursued advanced scientific studies at Cornell University; became a member of the faculty of Ohio State University; took up government work at the New York station; was later transferred to St. Louis, finally to New Orleans. His special interest as a chemist was in colors.

His early death deprives the Federal service of an efficient and much beloved executive officer and removes from this association one whose gifts promised unusual achievement.

The committee recommends the adoption of the following resolution:

*Resolved*, That this association suffers, in the early death of Albert L. Burns, the loss of a valued worker and an honored member.

Edwin F. Ladd, for many years connected with, finally head of, the Agricultural College of North Dakota, State Food Commissioner, in recent times United States Senator from that State, died during the past summer in his 66th year.

A native of New England, he was graduated from the University of Maine, became assistant chemist, then chief chemist, of the New York State Experiment Station and, later, made his way to the Northwest—where, until his death, he occupied a position of outstanding importance. So universal were his interests, so varied were his activities, so general was the confidence placed in him, that it would seem almost unthinkable that any matter of public concern that called for scientific study and treatment could make its appearance in North Dakota without Mr. Ladd's being called upon to assume control.

After his official withdrawal from what might be regarded as the scientific guardianship of his State, Mr. Ladd carried with him to the United States Senate a vast fund of information, secured at first hand, concerning the problems that have weighed upon the communities amid which he had long lived and worked.

Mr. Ladd was for many years an active member of this association. He was chosen to fill, in turn, every position of honor in the gift of this body. As referee and as president, he was known to all of us—as he was to his associates, later, in the Senate—as a vigorous, fearless “People's Advocate”, devoted to the interests of those whose difficulties he so well knew—the rural populations of the Northwest.

The committee recommends the adoption of the following resolution:

*Resolved*, That this association desires herewith to make record of its high estimate of the services rendered by Edwin F. Ladd and to express its profound sorrow at his death.

Charles L. Penny, Professor in the University of Delaware, is dead at the age of 63. After graduation at Bucknell University, he was for a while a faculty member at Pennsylvania State College; but, on the establishment of the Delaware Experiment Station in 1890, he removed to that State and devoted the rest of his life to its service. After holding

the post of State Chemist for a number of years, he accepted the headship of the Department of Chemistry in the University of Delaware. He is survived by a widow.

Dr. Penny's scientific work was chiefly devoted to the study of soils and fertilizers. For many years he attended regularly the meetings of this association. Many valuable suggestions and numerous reports of careful research were contributed by him during the period of his connection with this body. Since his withdrawal from Experiment Station work, the older members of this association have felt keenly the absence of his winning personality, and the loss of a comrade whose courtesy was as charming as his intelligence was impressive.

The committee recommends the adoption of the following resolution:

*Resolved*, That in the death of Charles L. Penny this association has lost a valued friend and associate.

The career of Edward George Proulx has been sympathetically described in a recent number of *The Journal* of this association. It, therefore, simply remains for this committee to recommend the adoption of the following resolution:

*Resolved*, That through the death of E. G. Proulx this association has lost an active, inspiring worker, and those of us who knew him, a sincere and worthy friend.

A fitting tribute to the memory of Guilford L. Spencer having been composed by the president of this association, the committee feels that it will suffice if it recommend the adoption by the association of the following resolution:

*Resolved*, That the death of Guilford L. Spencer removes one to whose interest and wise enthusiasm in considerable degree are attributed the successful beginnings and later growth of this association.

Charles D. Woods was in many respects the typical New Englander. Born in Maine; a graduate of Wesleyan University; a teacher of chemistry at Wesleyan and at Wilbraham Academy in Massachusetts, and of agricultural chemistry at the University of Maine; associated with the Connecticut Agricultural Experiment Station; for many years in charge of the Agricultural Experiment Station of Maine; later a technical adviser in agricultural science for the State of Massachusetts—practically his whole life's work was devoted to the improvement of the agricultural methods employed in the New England States. That he was regarded as one whose knowledge was wide and judgment sound was proved by his election to a number of positions of honor.

Dr. Woods was for many years active in the work of this association, in that of the Association of Dairy, Food and Drug Officials, and in that of the Association of Feed Officials. Of the last-mentioned organization he was the third president.

He was broad and sturdy, mentally as well as physically—one who immediately inspired confidence and, through a friendly, really jovial manner, drew towards him those whose shyness might have kept them apart.

The committee recommends the adoption of the following resolution:

*Resolved*, That this association desires to record an expression of its gratitude for the admirable spirit and conscientiousness of the work done by Charles D. Woods in the advancement of agricultural science in this country, and of its affection for a most worthy comrade.

The committee recommends, in addition, the adoption by the association of the following resolutions:

1. *Resolved*, That the foregoing resolutions, having to do with the deaths of A. L. Burns, Edwin F. Ladd, Charles L. Penny, Edward G. Proulx, Guilford L. Spencer, and Charles D. Woods, be printed in the proceedings of this association, and that a copy of the preamble and of the resolution be in each case sent to the family of our deceased friend and coworker.

2. *Resolved*, That this association extends to its honorary president, Harvey W. Wiley, its congratulations upon his health and vivacity at the opening of his 82nd year, its thanks for his witty and informing address, and its best wishes for his continued well-being.

3. *Resolved*, That this association desires to express to its president, C. A. Browne, its grateful appreciation of the skill with which he has wrought to prepare for and conduct the meeting which is now drawing to a close.

4. *Resolved*, That this association offers its sincere thanks to its secretary, W. W. Skinner, to Miss Marian E. Lapp, and to their assistants, for the highly successful plans they laid for the meeting of 1925.

5. *Resolved*, That this association gratefully recognizes that the present very satisfactory condition of the work of editing and publishing *The Journal* of the association is chiefly due to the unselfish labor and admirable judgment of the Chairman of the Board of Editors, R. W. Balcom, and his associates.

6. *Resolved*, That the recent issuance of the new edition of *Methods of Analysis*, A. O. A. C. once more directs our attention to the magnitude of the long-continued and most exhausting labor bestowed upon it by its editor-in-chief, R. E. Doolittle, and his coworkers, and calls forth an expression of our admiration and of our gratitude.

7. *Resolved*, That to R. E. Doolittle also, as Chairman of the Committee on Editing Methods of Analysis, the special thanks of this association are again due, and that to his energy is largely to be attributed the speedy conduct of the routine business of this meeting.

8. *Resolved*, That this association extends its thanks to R. W. Dunlap, the Assistant Secretary of Agriculture, for his courtesy in attending its meeting and greeting its members.

9. *Resolved*, That to the management of the Raleigh Hotel the thanks of this association are due, in recognition of the many courtesies extended during this annual meeting.

G. S. FRAPS,  
JULIUS HORTVET,

W. W. RANDALL.

*Committee on Resolutions.*

Approved.

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The Wednesday morning session continued until 1.30 p. m., when all the reports had been presented. No afternoon session was held. The proceedings for Monday and Tuesday, October 26th and 27th, will be published in Nos. 2, 3, and 4 of Volume IX.

## CONTRIBUTED PAPERS.

### FORMALDEHYDE IN CERTAIN MARINE PRODUCTS<sup>1</sup>.

By D. B. DILL and P. B. CLARK (U. S. Food and Drug Inspection Station, San Francisco, Calif.).

When formaldehyde is found in a foodstuff it is usually considered to have been added as a preservative. Since the use of formaldehyde as a preservative is almost universally prohibited by law, its natural occurrence or development in a foodstuff is a matter of great interest.

It is well known that smoked meats contain formaldehyde. Twenty samples of smoked meat were found by Ishio and Aoki (1919)<sup>2</sup> to contain one part of formaldehyde in from 10,000–50,000 parts of meat. In such concentration formaldehyde in smoked meat must be looked upon as having resulted from the smoking process. When present in much higher concentration its intentional addition is indicated.

The natural development of formaldehyde in canned crab meat was discovered by Ishida (1917)<sup>3</sup>. He adopted the following procedure in the preliminary investigation: 100 grams of the crab meat was introduced into a flask together with 200 cc. of water, vigorously shaken, and allowed to stand overnight. The mixture was filtered, and the filtrate was placed in a 500 cc. distilling flask, together with 10 cc. of strong phosphoric acid. Six successive 20 cc. portions of distillate were collected and examined by each of several sensitive tests. The same procedure was carried out, 60 per cent alcohol being used as a solvent. Higher values for formaldehyde were obtained when water was used. Fifty or sixty commercial packs of crab meat were examined, for the most part with positive results. Ten tests for formaldehyde were employed. With the exception of the Leach test, all the tests described in the methods of the Association of Official Agricultural Chemists<sup>4</sup> were included. The fuchsin-sulfurous acid and the resorcinol tests were used also.

Ishida then prepared an authentic pack. Live crabs were boiled in water for 30 minutes. The meat was removed and tested as previously described. Weakly positive results were obtained. The remainder of the meat was canned and examined from time to time. After 8 months distinct tests for formaldehyde were obtained by all the more sensitive tests. Ishida's finding seems to be the first record of the wholly natural

<sup>1</sup> This investigation was initiated at the Seattle Food and Drug Inspection Station under the direction of A. W. Hansen and completed at the San Francisco Food and Drug Inspection Station. C. L. Alsberg has offered invaluable advice.

<sup>2</sup> *J. Pharm. Soc. Japan*, 1919, 443: 20-40; *C. A.*, 1919, 13: 982.

<sup>3</sup> *Ibid.*, 1917, 422: 300.

<sup>4</sup> *Methods of Analysis*, A. O. A. C., 1925, 132.

development of formaldehyde, or of a substance giving the formaldehyde reaction, in a foodstuff. His investigation was confined to crab meat.

The natural development of formaldehyde in a sterile and enzyme-free food product is of great interest to those charged with enforcement of the pure food laws. It is also of special interest to students of photosynthesis on account of the formation of formaldehyde from carbon dioxide and water vapor through the agency of ultraviolet light. While Baly and his co-workers (1921)<sup>1</sup> claim that such a reaction takes place, Spoehr (1923)<sup>2</sup> was unable to verify it when all organic matter was excluded from the reaction tube. Neither were Porter and Ramsberger (1925)<sup>3</sup> able to reduce pure carbon dioxide and water vapor to formaldehyde by ultraviolet light.

The authors have made a number of experimental packs of various marine products during the past three years. These and a large number of commercial packs have been examined with the hope of answering at least partially the following questions:

- (1) Is this development of formaldehyde related to incomplete sterilization?
- (2) Is it restricted to canned crab meat?
- (3) Is it dependent on the nature of the container?
- (4) Is it related to can corrosion or to the blackening process?
- (5) Can the substance be definitely identified as formaldehyde?
- (6) What concentration of formaldehyde is developed?
- (7) In case the substance can be identified as formaldehyde what is its mother substance?

#### EXPERIMENTAL.

During the fall and winter of 1922 experimental packs of crab meat and shrimp meat were prepared. Live Dungeness crabs (*Cancer magister*) were boiled for 30 minutes, cooled, and shelled. Part of the meat was tested for formaldehyde (as described below), and the remainder was canned.

The shrimp meat (species undetermined) was obtained from a fish dealer, who received it direct in five pound "seal-ship" cans from Alaska. It had not been sterilized but had been in cold storage, and it appeared to be in excellent condition. It was packed in the same manner as the crab meat, plain cans, lacquered cans, lacquered cans with parchment paper lining, and one-half pint glass jars with glass lids being used. Sterilization was effected by heating in steam for 30 minutes at 100°C. before sealing and for 90 minutes at 115°C. after sealing.

In the spring of 1924, a pack of California spiny lobster (*Panulirus interruptus*) was prepared. Live lobsters were obtained and handled in the same way as the crabs. At the same time several pounds of the brine shrimp (*Artemia salina*), a minute phyllopod from brine ponds

<sup>1</sup> J. Chem. Soc., 1921, 119: 1025.

<sup>2</sup> J. Am. Chem. Soc., 1923, 45: 1184.

<sup>3</sup> Ibid., 1925, 47: 79.

near Redwood City, California, were packed. This small crustacean is representative of the food of many species of fish.

In the examination of these products for formaldehyde, 100 grams of the ground flesh was washed into a round bottom liter flask with 100 cc. of 2 per cent  $H_3PO_4$  and then steam distilled while being heated in a salt bath under such conditions as to keep the volume approximately constant. The first 50 cc. of distillate was employed for the formaldehyde test. Three of the qualitative tests described in *Methods of Analysis*<sup>1</sup> were used. These were the phenylhydrazin hydrochloride and sodium nitroprusside test, the Leach test, and the phenylhydrazin hydrochloride and potassium ferricyanide test. The result was classed as positive when a bright red color was obtained with the last of these three methods. Results classed as positive by this test were usually positive, although sometimes doubtful or negative, by the Leach test and were frequently doubtful or negative by the less sensitive nitroprusside test.

It can be stated definitely that this development of formaldehyde is not related to the growth of organisms. The conditions of sterilization were such as to insure the destruction of all bacteria and bacterial spores. Not a single container of the experimental packs used by the writers has exhibited any sign of non-sterility. The cans have not leaked, and there has been no evidence of gas formation or odor of putrefaction. Yet, in confirmation of the finding of Ishida (1917), the experimental crab meat pack gave a distinctly positive test for formaldehyde a few months after canning.

It has been found that this natural development of formaldehyde is not restricted to canned crab meat. All the experimental packs of other crustacea gave positive tests for formaldehyde a few months after canning. Commercial packs of lobster (various species), crab (*Cancer magister* and *Paralithodes camtschatica*) and shrimp (various species) give positive tests. It is interesting to note that the so-called red rock cod of the Pacific coast (*Sebastes* sp.) gave a strongly positive test six weeks after canning.

All three of the tests for formaldehyde previously mentioned were negative when applied to commercial packs of the California sardine (*Sardinia caerulea*), pink salmon (*Oncorhynchus gorbuscha*), long-fin tuna (*Germo alalunga*), and the Washington clam (*Paphia staminea*). Negative tests were obtained from experimental packs of chum salmon (*Oncorhynchus keta*), sockeye salmon (*O. nerka*), barracuda (*Sphyræna argentea*), and the mackerel (*Scomber japonicus*).

A commercial pack of herring (*Clupea pallasii*) gave a positive test. This was lightly smoked, and yet an experimental pack of the closely related sardine (*Sardinia caerulea*) that had been smoked gave a nega-

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 132.



tive test. The herring evidently had been poorly cleaned, and it seems quite possible that the formaldehyde originated from the food, which is largely crustacean in character. Support is given this suggestion by the fact that the experimental pack of the phyllopod *Artemia salina* developed formaldehyde.

The nature of the container was thought to be a possible factor in this phenomenon. Crustacea are usually packed in cans that are lacquered or lined with parchment paper or sometimes both lacquered and lined with parchment paper. Formaldehyde developed in the experimental packs used in this work, in all the containers, whether metal or glass. Lacquer, parchment paper, and tin, therefore, can not be involved.

The phenomenon is not related to the can-corrosion ability of crustacea; neither is it related to the fact that the flesh of canned crustacea may turn black from the formation of iron sulfide when packed in lacquered or plain cans. Barracuda (*Sphyræna argentea*) and the red rock cod (*Sebastes* sp.) corrode cans rapidly, but while formaldehyde develops in the cod within a few weeks, it has never been found in experimental packs of the barracuda although one of these packs was three years old and the cans were badly corroded. While *Sebastes* corrodes cans rapidly, it has never been observed to blacken in the can; the flesh retains its normal color. Hence the tendency of crustacea to blacken is independent of formaldehyde formation.

Typical formaldehyde reactions have been obtained not only by the three tests named previously but also by the Hehner, the phloroglucinol, and the Schryver tests. All the tests employed gave wholly typical color reactions with the exception of the sodium nitroprusside test, which was frequently inconclusive, giving a browner color than a dilute solution of pure formaldehyde. The evidence is conclusive that the substance in the distillate actually is formaldehyde. The question may be raised as to whether or not the formaldehyde is formed during the process of distillation. It is probably true that the formaldehyde does not exist free in these products; however, it is loosely enough bound so that the liquor from the canned product frequently gives a positive Leach test.

An effort was made to determine the approximate amount of formaldehyde present in these products. The distillate from a commercial lobster pack was found to give no appreciable reduction of mercuric chloride, indicating the absence of formic acid. The method followed in examining for formic acid consisted simply in rendering about one liter of the distillate weakly alkaline with sodium hydroxide, evaporating to small volume, strongly acidifying with strong phosphoric acid, and distilling. The distillate was then employed for the quantitative determination of formic acid by the A. O. A. C. method<sup>1</sup>. Having established the absence of formic acid in this pack, 3 liters of distillate, representing about 1200

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 137.

grams of meat, was obtained. The distillate was treated with 5 grams of sodium peroxide and evaporated to small volume. After acidification and distillation, formic acid was determined. An abundant precipitate of mercurous chloride was obtained, its weight indicating that if all the formaldehyde was obtained from the meat it had been present to the extent of about one part in 100,000. Incidentally it may be mentioned that a 0.1 per cent solution of methyl alcohol was not oxidized to formic acid under the above conditions.

When formaldehyde was added to canned salmon in the proportion of one part in 20,000 and one part in 100,000, all of it could not be recovered by acidification and steam distillation. Even when 200 grams of such samples was treated with 300 cc. of 2 per cent phosphoric acid and distilled for six hours, giving about 2500 cc. of distillate, the distillate still continued to give slight tests for formaldehyde. Estimation of the amount of formaldehyde in the distillate by comparison with standardized formaldehyde solutions, employing Schryver's test, indicated that from the sample containing one part in 20,000 of formaldehyde only about one-third of the formaldehyde was recovered. From the sample containing one part in 100,000 about one-fifth was recovered.

Hope, therefore, was abandoned of obtaining an exact quantitative measure of the formaldehyde present in canned crustacea. Several determinations were made, the results of which are shown in the accompanying table. The method followed was that just described, Schryver's test and standardized formaldehyde solution being used. Schryver's test was carried out as follows: To 10 cc. of the sample was added 0.1 gram of phenylhydrazine hydrochloride. When this was dissolved, there were added one cc. of 5 per cent potassium ferricyanide solution and, after one minute, 3 cc. of strong hydrochloric acid. This was then diluted with 20 cc. of water and extracted in a separatory funnel with 20 cc. of ethyl ether. The ether extract was extracted with 5 cc. of strong hydrochloric acid. The acid extract was diluted to 25 cc. with 1:1 hydrochloric acid. Comparison was then made with the color developed from standard formaldehyde solution. The color is not permanent, and comparison must not be delayed. The results shown in the table are the concentrations actually found. The experiments with salmon described previously suggest that the actual concentrations of formaldehyde were from three to five times those reported in the table.

No progress has been made on the study of the origin of the formaldehyde. Since its development takes place in the absence of oxygen and frequently in the presence of ferrous sulfide, it can hardly be formed by an oxidizing reaction. One thinks of the possibility of its formation by the reduction of formic acid. There has not been opportunity to make a careful investigation of the possible occurrence of formic acid in fresh crustacean flesh. It is well known that formaldehyde is an oxidation

product of chlorophyll. It is suggestive that the sculpin, *Sebastodes*, is highly colored (hence the name, red rock cod), and it is possible that its pigment is similar to that of the crustacea. No investigation has been made of the possible origin of formaldehyde from a common pigment.

*Formaldehyde obtained from canned crustacea by acidification and steam distillation.*

	FORMALDEHYDE OBTAINED
California spiny lobster ( <i>Panulirus interruptus</i> ).....	1 : 65,000
California spiny lobster ( <i>Panulirus interruptus</i> ).....	1 : 45,000
South African spiny lobster (unidentified).....	1 : 40,000
Shrimp (unidentified, from Gulf of Mexico).....	1 : 35,000
Canadian lobster ( <i>Homarus americanus</i> ).....	1 : 30,000
Canadian lobster ( <i>Homarus americanus</i> ).....	1 : 100,000
Spider crab ( <i>Paralithodes camtschatica</i> ).....	1 : 72,000
Spider crab ( <i>Paralithodes camtschatica</i> ).....	1 : 200,000

### SUMMARY.

Formaldehyde develops in sterile canned crustacea and so-called red rock cod (*Sebastodes* sp.).

This development of formaldehyde is independent of the nature of the container and is independent of the processes of can corrosion or of the blackening of these products from iron sulfide formation. It takes place in the absence of free oxygen.

Acidification and steam distillation failed to recover more than one-third the formaldehyde added to formaldehyde-free salmon. It is shown that the concentration of formaldehyde in canned crustacea is sometimes as high as one part in 30,000, and if one takes into account the failure to secure complete recovery in distillation, it is possibly as high as one part in 10,000.

## THE QUANTITATIVE DETERMINATION OF UNSAPONIFI- ABLE MATTER IN WHEAT FLOUR, ALIMENTARY PASTES, AND EGGS.

By RAYMOND HERTWIG and L. H. BAILEY (Bureau of Chemistry, Washington, D. C.).

The unsaponifiable matter of wheat flour<sup>1</sup>, wheat products<sup>1</sup>, alimentary pastes<sup>2, 3</sup>, and eggs<sup>4</sup>, has been determined in the past almost solely in the residue of the ethyl ether or petroleum ether extract after the removal of the ether.

Some years ago it was shown by Hertwig<sup>5</sup> that direct extraction with

<sup>1</sup> Thesis: A Study of Wheat Oil, submitted to the Graduate Faculty of the University of Minnesota by C. D. Ball, 1924.

<sup>2</sup> I. König. *Chemie Menschlichen nahr. Genuss.*, 1914, 4 Auflage, III, Band, 2. Teil., pp. 665-6.

<sup>3</sup> E. Abderhalden. *Handbuch biolog. Arbeitsmethoden*, 1922, Abt. IV, Teil. 8, Heft 1, pp. 287-9.

<sup>4</sup> *Ibid.*, Heft II, pp. 531-2.

<sup>5</sup> *This Journal*, 1923, 6: 508.

ether does not obtain all the ether-soluble and fat-like substances from wheat flour, alimentary pastes, and eggs. He proposed two methods by which greater quantities of such substances may be extracted—an acid hydrolysis method, and a “neutral” method, i. e. one using neutral extracting agents<sup>1</sup>.

The “neutral” method gives higher results than those obtained by the acid hydrolysis procedure, which destroys the lecithins and related phosphatides. Rask and Phelps<sup>2</sup> proposed a method involving the use of alkaline extracting agents to determine lipoids in flour and alimentary pastes. Their method yields results quite similar to those obtained by the Hertwig “neutral” method. Therefore the assumption seems justified that the published methods for the determination of unsaponifiable matter in the products in question give low results since the preliminary ether extract obtained by these methods does not contain all the unsaponifiable matter.

In connection with certain official food regulatory work, it is a matter of importance to know whether the usual method of direct ether extraction enables the quantitative determination of unsaponifiable matter in the products under consideration. Consequently a comparative study was undertaken involving the determination of the unsaponifiable matter contained in extracts obtained by the various methods mentioned in this paper from certain samples of flour, alimentary pastes, and eggs. Petroleum ether extract was not included with the others as it is generally recognized that this extracting agent is not so efficient as ethyl ether. The unsaponifiable matter in the respective extracts was determined by the Kerr-Sorber method as modified by Hertwig and Jamieson<sup>3</sup>. This method was used because of its simplicity, its proven excellence as a quantitative method, and because it gives results practically identical with those of the somewhat more laborious method adopted as official at the 1925 meeting of the Association of Official Agricultural Chemists (p. 45).

The results obtained are given in the table.

The data in the table indicate that the direct ether extract of flour and eggs does not contain all the unsaponifiable matter of these materials. The hydrolysis of the sample with strong acid in the acid hydrolysis method for extraction of fat apparently has some chemical action on the unsaponifiable matter which causes low results for flour and eggs. The A. O. A. C. neutral method for the lipid extraction of flour, alimentary paste, and eggs gives the highest results. This method is also believed to be the most satisfactory from the standpoint of operation for general purposes, and it is also, to all appearances, the most accurate quantitatively.

<sup>1</sup> *This Journal*, 1923, 7: 91.

<sup>2</sup> *Ind. Eng. Chem.*, 1925, 17: 189.

<sup>3</sup> *This Journal*, 1925, 8: 439.

*Unsaponifiable matter.*

MATERIAL	DIRECT ETHER EXTRACTION	RASK-PHELPS METHOD	ACID HYDROLYSIS METHOD	NEUTRAL EXTRACTION METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Flour Sample (a) . . . . .	0.20 0.23	0.30 0.32	0.16 0.21	0.29 0.33
Flour Sample (b) . . . . .	0.20 0.30	0.30 0.33	0.14 0.15	0.41 0.43
Flour Sample (c) . . . . .	...	...	0.20 0.22	0.31 0.32
Com'l Dried Yolk (a) . . . . .	3.04 3.05	...	3.17 3.19 3.27 3.42	3.63 3.64
Com'l Dried Yolk (b) . . . . .	...	...	3.07 3.08	3.32 3.50
Yolk Noodle . . . . .	...	...	0.61 0.66	0.66 0.69

The unsaponifiable matter obtained from all the samples and by using all the methods responded very satisfactorily to the chloroform sulfuric acid color test for plant and animal sterols<sup>1</sup>.

The following method is recommended for the quantitative determination of unsaponifiable matter in wheat flour, alimentary pastes, and eggs.

*Method.*

## A. Extraction of the lipoids.

*For wheat flour and alimentary paste:* Extract the lipoids from 5 grams of sample according to the A. O. A. C. method for flour adopted at the 1925 meeting (p. 40). Use either the dried crude lipoids or the final purified and weighed lipoids for the determination of the unsaponifiable matter.

*For eggs:* Extract the lipoids from 10 grams of liquid eggs or 2 grams of powdered dried egg according to the A. O. A. C. method for lipoids in eggs<sup>2</sup> (p. 58). Use either the dried crude lipoids or the final purified and weighed lipoids for the determination of the unsaponifiable matter.

## B. Determination of unsaponifiable matter.

*For wheat flour, alimentary pastes, and eggs:* Add 30 cc. of alcohol and 3 cc. of concentrated potassium hydroxide (1+1) to the lipoids as obtained above and proceed according to the method for unsaponifiable matter<sup>3</sup>. Preferably weigh the unsaponifiable matter in a light weight 100 cc. capacity flask.

<sup>1</sup> Haas and Hill. *Chemistry of Plant Products*, 1913, p. 17.

<sup>2</sup> *This Journal*, 1925, 8: 602.

<sup>3</sup> *Ibid.*, 441.





GUILFORD L. SPENCER, 1858-1925

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## DR. GUILFORD L. SPENCER

### A PERSONAL TRIBUTE

by

Harvey W. Wiley

Dr. C. A. Browne, in the July, 1925, issue of *The Reference Book of the Sugar Industry of the World*, has given a full account of the scientific activities of Dr. Guilford L. Spencer. It falls to my lot to pay a more personal tribute to my pupil of long ago and life-long friend.

Purdue University opened its doors for regular instruction in September, 1874. The previous spring, in order to comply with the conditions of the location of the school near Lafayette, Professor Hougham held a school with a few pupils in one of the buildings of the university that had been completed. I attended the first regular faculty meeting a few days before the opening of the school. It was a motley but small group of students that gathered there on the first day; in all, about thirty-seven young men of different degrees of instruction, and mostly from the farms, appeared at the chapel exercises. There was a fair sprinkling of boys, however, from Lafayette, and among them was a slender youth who wanted to study chemistry. I immediately took him into the laboratory and put him to work. I believe I had him make sulfate of zinc and burn the hydrogen that was produced as his first experiment. He received the precautions necessary to prevent an explosion. It is interesting, in this connection, to recall that at the Centennial Exposition at Philadelphia in 1876, a collection of inorganic chemicals made by the students of Purdue University was shown. I believe this was the first collection of its kind ever made. Spencer was a liberal contributor to this project.

Young Spencer took to chemistry as a duck does to water; it was his natural element. He became the most enthusiastic student in the laboratory and progressed rapidly, not only in his chemical studies, but in all others that were included in his course. During this time I was engaged largely in the examination of sugars and sirups and of sugar-producing plants. On my father's farm in southern Indiana were sugar maple trees, and there was one particular tree which we called the "sweet tree". The woodpeckers were excellent chemists, and they filled that tree with holes every spring for the purpose of getting at the sap within. As they could not collect all the sap which exuded from these holes, it trickled over the bark and made it very black, so that the tree was quite distinct in color from its fellows in the sugar bush. I was interested to know just how much sugar there was in the sap of this tree, as compared with the others, so one of the things I did while Spencer was my student was to bring samples of sap from several trees for analysis. It was found that the woodpeckers had excellent judgment of chemical composition, as the quantity of sugar in the sap of this particular tree was much larger than in that of any of the surrounding trees.



Young Spencer early acquired a taste for a sugar career and desired to fit himself as a sugar engineer. Although Purdue was a technical school, its engineering courses were extremely feeble during the first few years of its existence. I, therefore, advised him to go to Ann Arbor for a year in engineering in order to prepare himself for more than the chemical control of a sugar factory. This he did, and took his Master's Degree from the University of Michigan in 1882. His enthusiasm was toward the beet-sugar rather than the cane-sugar industry. Since there were no cane-sugar factories in the United States at that time that had any form of chemical control, he went to France for a period of two years, bearing letters to friends of mine engaged in the beet-sugar industry. He formed friendships there quite as dear to him and as lasting as those he had formed at Purdue and elsewhere, as indicated by the item in Dr. Browne's memoir recording Spencer's meeting with Pellet many years after he had been associated with him in the sugar-beet industry in France.

During my studies in Germany in 1878, I became more interested than ever before in matters pertaining to sugars, and especially, of course, to beet sugar. When I assumed my duties as Chief of the Division of Chemistry in 1883, it was largely through my work and papers relating to sugars that I was selected for this position. At that time the Department of Agriculture was engaged in a very extensive investigation of sorghum as a possible sugar-producing plant. My relations to sorghum were rather intimate. In the late 1850's my father received from Representative William McKee Dunn, our member of Congress from Indiana, a package of seeds which looked like broom corn seeds, labeled "*Sorghum Saccharatum*". My father had little interest in the matter, but I, as a boy, planted the seeds and watched them as they grew. They were very puny-looking plants as they came up, but soon they became more vigorous; later on tassels came out, bearing seeds like broom corn. Towards the end of the season these seeds gradually turned black. As the stalks matured, I selected one of them, took it to the house, cut off the tough outside, and put a piece of the exposed pithy inner portion into my mouth. I was delighted to find it extremely sweet. With the aid of my father, or brother—I have forgotten which—I twisted this stalk until almost a pint of liquid was obtained. I put this on the stove, and within a short time I had made some sorghum sirup, the first, I think, ever made in southern Indiana.

I was impressed with Dr. Peter Collier's work in Washington in the promotion of the sorghum-sugar industry, although I did not know him personally. There were many other persons, likewise, interested in the matter, among them two young professors in the University of Illinois, at Champaign, which is only forty miles west of Purdue. I went over there to interview Professor Weber and Professor Scovell, as I had become imbued with the idea that sorghum at least would be a useful plant for the manufacture of sirup. I organized among my friends in Lafayette a small corporation for the production of sorghum sirup. (This was two or three years before I was called to Purdue.) We made something of a financial success of our venture the first year, so that when I was called to Washington and wished to dispose of my stock my colleagues were willing to buy it. Unfortunately, within a year or two afterward, the factory, which we had built some few miles away from Lafayette, was

struck by lightning and burned. This was the end of the sorghum industry in that part of the country.

As I was eager to continue the investigation and to have my former pupil with me, upon his return from France Dr. Spencer became a member of the Bureau of Chemistry, where he had opulent opportunity to practise his profession. We soon branched out into wider fields and inaugurated the first exhibit of a sugar laboratory ever presented to an exposition in 1884. It was through this exhibit that the sugar planters of Louisiana were induced, the next year, to organize a sugar experiment station and to place at its head that eminent investigator, Dr. W. C. Stubbs, who recently passed away.

The first sugar plantation to become interested in chemical control in Louisiana was that of Governor H. C. Warmoth, at Magnolia, some 40 or 50 miles south of New Orleans. In fact, it was the last plantation before the jetties. Spencer was assigned to do the experimental work in establishing sugar control at Magnolia. He had already met Governor Warmoth, a man of wonderful foresight and enthusiasm, and he and Dr. Spencer remained firm friends throughout life. Spencer's acquaintance with the Governor also had much to do with his future work. Warmoth went to Louisiana after the Civil War. He became governor in the early days of reconstruction; by his wisdom, tact, and energy he won the respect and confidence of the best people in the State, and retained them when the political power in the State reverted to the white citizens. In 1884 Warmoth, who realized that the planters were losing a large percentage of the sugar in the cane, decided to go to Europe to study the method of extracting the sugar from sugar beets. He came to Washington and applied for help to the Department of Agriculture. The Commissioner of Agriculture sent for Spencer, and arrangements were made by which Spencer was commissioned to go with Warmoth to France. To this incident is due the subsequent experiments which led to the introduction of diffusion at Magnolia. This experiment increased the yield of sugar by nearly 40 per cent and led to the immediate improvement of the cane mills, with a permanent increase in the output of sugar.

Spencer owes to the Magnolia plantation another debt. There he met an eminent engineer and inventor, Samuel Fiske, who was engaged in installing one of his inventions, a cane shredder, the purpose of which was to secure a higher percentage of sap. Due to this acquaintance, he subsequently met Mr. Fiske's daughter. The most natural thing in the world happened when an able young chemist and a charming girl met. Miss Fiske became the ideal wife and the inseparable companion of her distinguished husband. Fortunately, she was with him at the time of his tragic death, which had long been anticipated. Dr. Spencer in his last years disclosed the true heroism which always guided him. His set purpose was to die in harness; he was never driven from his purpose to give all his service to his career.

Although Spencer became more and more occupied in Louisiana and in the tropics, I still held on to him in the Bureau of Chemistry, giving him leave of absence to the fullest extent desired by him for the administration of sugar control in Louisiana. He was eventually called to the tropics where meteorological conditions were much more favorable to the development and maturity of the sugar cane. Spencer's association with me in

the experimental work which I conducted on sugar cane in Louisiana and on sorghum in Kansas remained unbroken during almost its whole progress. Experimental factories were built in Louisiana, in Kansas, and in Florida. Spencer was the leader and conductor of all this chemical sugar engineering. He worked efficiently, faithfully, and enthusiastically for many years as my right-hand man in all matters pertaining to sugar engineering and sugar control. These experimental investigations were brought to a close on the accession of J. Sterling Morton as Secretary of Agriculture. Secretary Morton was bitterly opposed to doing much experimentally for the farmer. He thought farming, like other business, ought to attend to its own experiment investigation. To that end, without consulting me, he ordered all these experiment stations closed and the expensive machinery installed therein sold as junk. This freed Spencer from any further investigations of this kind and opened the way for a wider field in sugar engineering, which he soon entered.

My personal interest and admiration for Dr. Spencer increased with his advancing years and his growing reputation. In all of his successes I felt an integral part, just as every teacher loves to see a pupil's success. But Spencer more than succeeded. He commanded. He reached the highest pinnacle in his profession.

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It is with profound sorrow that we announce the death of Mr. R. E. Doolittle, who for many years had been a member and one of the most active workers of this association. His health recently had not been good. He collapsed in his office on the afternoon of Friday, the twenty-third of April, and died at his home in Evanston, Ill., the following Sunday.

Mr. Doolittle was president of the association in 1924 and for a number of years had been chairman of two of its most important committees, the Committee on Recommendations of Referees and the Committee on Editing Methods of Analysis, as well as a member of the Board of Editors of *This Journal*. The news of Mr. Doolittle's sudden passing will bring a sense of grief and of great loss to all members of the association.

An obituary will appear in a later issue of the *Journal*.

BOARD OF EDITORS.

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## FIRST DAY. MONDAY—MORNING SESSION.

### REPORT ON WATERS, BRINE, AND SALT.

By C. H. BADGER (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

Last year the association recommended that the Referee on Waters, Brine, and Salt arrange for collaborative work on the new method for the determination of hydrogen sulfide<sup>1</sup> described in the report for 1924. In accord with this recommendation, solutions for analysis were prepared by adding water saturated with hydrogen sulfide to one-gallon portions of distilled water in demijohns. The solutions were then made neutral, acid, or alkaline by the addition of definite quantities of acid or alkali, phenolphthalein being used as indicator. The neutral solutions were made by adding approximately 0.5 *N* sodium hydroxide solution, followed by one drop of dilute hydrochloric acid (1 + 1) to destroy the pink color of the indicator; the acid solutions were made by adding 1.7 cc. of strong hydrochloric acid; and the alkaline solutions were made by adding 10 cc. of 10 per cent sodium hydroxide in three instances and 15 cc. in the fourth. All the samples were carefully siphoned from the demijohns, care being taken to avoid agitation.

The samples were analyzed by the old official method<sup>2</sup>, which was abandoned by the association last year, and by the new method described in the referee's report for 1924. Four chemists, all members of the Water and Beverage Laboratory of the Bureau of Chemistry, cooperated in the work. The results obtained are given in Table 1.

#### DISCUSSION OF DATA IN TABLE 1.

Because of the rapid change in the content of hydrogen sulfide which takes place in hydrogen sulfide solutions, it was not practicable for the collaborators to analyze the same solutions. They, therefore, prepared their own solutions in the manner which has been described and then analyzed them at once. Moreover, because of the character of the samples, there is no absolute standard of comparison with regard to actual content of hydrogen sulfide.

The new method gave closely agreeing results on all types of solutions. The results in triplicate, obtained by the old method, did not agree very

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<sup>1</sup> *This Journal*, 1925, 8: 332; 1926, 9: 29.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 93.

well on most of the samples and in the case of the alkaline solutions, pH 11.3, 11.5, and 12.0, varied so widely as to be worthless.

With the neutral and acid solutions, the results by the new method are higher than by the old method. With the alkaline solutions no comparison of the results obtained by the two methods is possible, because those obtained by the old method are inconsistent; they also confirm results previously found and definitely show that this method is not worthy of any further consideration.

It is not necessary to neutralize acid samples since the results obtained by the new method without neutralization are practically the same as those obtained when the samples were neutralized with sodium hydroxide. Last year slightly lower results were obtained with acid samples when sodium hydroxide was omitted, but the referee has concluded from tests that he has conducted that the slightly lower results were due to the loss of hydrogen sulfide from the samples.

It is necessary to neutralize alkaline samples as such samples without neutralization give abnormally high results by the new method.

The referee and the collaborators are of the opinion that the new method is superior to the old method for the following reasons:

1. It is more rapid. In the new method a definite volume of the sample can be measured quickly and titrated, while in the old method the measurement of the sample requires considerable time with possibility of loss of hydrogen sulfide.

2. Calculations are made more readily in the new method. In the old method, for example, one analyst calculated the milligrams per liter of hydrogen sulfide from the following size samples, 7.55 cc., 5.85 cc., and 5.40 cc.

3. The results of triplicate experiments by the new method are more concordant.

4. The new method is more accurate, especially with samples containing substantial quantities of hydrogen sulfide, because larger samples can be taken.

5. It is impracticable to comply with the direction in the old method to "add the water under examination until the color of the iodine disappears", because the end point is uncertain.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That the method for the determination of hydrogen sulfide in waters be dropped (final action).

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 70.

(2) That the new method be made official (second presentation).

(3) That the referee for next year study methods for the analysis of salt with particular reference to the determination of ingredients that are added to prevent caking.

TABLE 1.

*Comparison of methods for the determination of hydrogen sulfide.*

(100 cc. samples used and results expressed as milligrams per liter.)

COLLABORATOR	A. E. MIX			J. B. WILSON			C. E. GOODRICH			C. H. BADGER		
SOLUTION NO.	1	2	3	4	5	6	7	8	9	10	11	12
pH Value*	7.6	2.4	11.3	7.3	2.5		7.5	2.3	11.5	7.5	2.4	12.0
Old Method	19.4	26.3	30.8	36.5	51.1	44.3	21.3	35.0	42.6	24.1	32.7	70.1
	20.6	24.5	36.6	39.0	49.1	59.0	22.6	34.9	58.1	24.0	31.0	57.8
	19.4	23.5	29.6	37.9		54.1	23.3	33.5	64.9	25.1	31.0	55.9
New Method	39.6	56.5	49.1	47.6	58.2	46.1	39.6	51.1	51.4	40.4	49.4	14.2
	39.6	55.2	49.1	47.4	58.2	46.2	40.3	51.4	51.4	40.1	49.1	14.0
	39.6	56.5	49.1	47.7	58.1	46.1	39.3	49.7	52.1	40.1	48.9	14.2
		55.5						49.4			48.7	
		55.5						49.7				
		55.5										
New Method†		55.2	80.8		58.4	78.1		49.7	86.4		49.6	38.9
		55.2			58.4			51.1			49.4	
		54.5			59.7			51.1				

\* pH value of samples before analysis.

† Samples were not neutralized before analysis.

*F. P. Veitch:* Mr. President, Mr. Frey has asked me to report that no collaborative work has been carried on this year. The methods of the association on tanning materials and leathers were thoroughly revised for the 1925 edition of *Methods of Analysis*. There have been no developments during the past year in analytical methods that required collaborative study, except possibly an application of the Bidwell-Sterling distillation method for the determination of moisture. Since, however, the association has a referee on this subject, it was thought to be desirable to await this report. Their modification of the old toluene distillation method may be particularly applicable to tanning materials and leathers.

## REPORT ON INSECTICIDES AND FUNGICIDES.

By J. J. T. GRAHAM (Insecticide and Fungicide Laboratory, Bureau of Chemistry, Washington, D. C.), *Referee*.

Following the recommendations contained in the report of the referee, the work on insecticides and fungicides for 1925 consisted of a study of

methods for the analysis of mineral oil-soap emulsions and a comparison of the xylene distillation method with the official method for water in soaps. Seven chemists agreed to assist in the work, and samples were prepared and sent to them.

#### MINERAL OIL-SOAP EMULSIONS.

##### PREPARATION OF SAMPLES.

*Sample 1.*—Prepared from a red engine oil, potash fish oil soap, and water, according to the formula given by Ackerman<sup>1</sup>.

*Sample 2.*—Prepared from kerosene, soda fish oil soap, and water, according to the formula given by Quaintance and Siegler<sup>2</sup>.

In each case the oil was heated to about 90°C., and the soap was dissolved in water by heating nearly to boiling. The soap solution was then poured into the oil, and the mixture was pumped four times through a spray pump. The emulsions obtained were of good quality and showed no tendency toward separation of oil, though creaming out occurred after long standing.

The methods of analysis sent out by the referee are as follows:

#### METHODS OF THE DIVISION OF CHEMISTRY, CALIFORNIA STATE DEPARTMENT OF AGRICULTURE, FOR THE ANALYSIS OF MINERAL OIL-SOAP EMULSIONS.

##### WATER.

Weigh about 50 grams of the sample into a copper retort. Distil slowly, receiving the distillate in a standardized tall graduated cylinder. Read the volume of the water layer at the bottom of the cylinder, and from this reading calculate the percentage of water in the sample.

It is often advisable to add xylene or kerosene to assist in bringing over the water and to prevent foaming.

##### TOTAL OIL.

(Modification of "Determination of Kerosene in Kerosene Emulsions"<sup>3</sup>.)

Weigh about 10 grams of the sample into an 18 gram Babcock cream bottle. Dilute with about 10 cc. of hot water and add 5-10 cc. of dilute sulfuric acid (1 + 1).

Set the bottle in a hot water bath for about 5 minutes to hasten the separation of the oil, add sufficient saturated sodium chloride solution to bring the oil layer within the graduations on the neck of the bottle, whirl at a rate of 1200 revolutions per minute for about 2 minutes, and allow to cool. Read the volume of the oily layer, determine its density, and from these values calculate its weight and percentage. From this percentage value, deduct the percentage of fatty acids (and phenols if present) determined separately.

<sup>1</sup> U. S. Dept. Agr. Circ. 263, p. 13.

<sup>2</sup> U. S. Dept. Agr. Farmers Bull. 908, p. 28.

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 105, p. 165.

## SOAP.

*Method I.*

Evaporate about 10 grams of the sample in a platinum dish; ignite to a white ash; weigh; and titrate with 0.1 normal hydrochloric acid, using methyl orange as indicator. From the number of cc. of hydrochloric acid used, calculate the percentage of sodium or potassium stearate or rosin soap.

*Method II.*

(Modification of a method by J. Marcusson<sup>1</sup>.)

Weigh 20 grams of the sample into a separatory funnel, add 60 cc. of petroleum ether, and extract the mixture 5 or 6 times with 10 cc. portions of 50 per cent alcohol. Combine the alcoholic extracts, wash once with petroleum ether, and evaporate on a steam bath to remove alcohol. Dissolve the residue in about 100 cc. of water made alkaline with sodium hydroxide.

Cool and precipitate the soap by adding an excess of saturated sodium chloride solution with vigorous stirring. After the precipitate of soap is well coagulated, filter and wash with a saturated sodium chloride solution.

Punch a hole in the point of the filter and wash the precipitate back into the beaker in which the precipitation was made, and wash the filter paper thoroughly with hot water. Cool the solution, acidify with hydrochloric acid, and extract three times with 20 cc. portions of ether.

Wash the ether fractions once with water, collect them in a weighed beaker, evaporate the ether on a steam bath, and weigh as fatty acids.

(As an optional method, instead of weighing the fatty acids they may be dissolved in alcohol and titrated with 0.1 normal alkali.)

From the percentage of fatty acids, calculate the percentage of soap in the sample as sodium or potassium stearate.

Rosin in the soap may be detected by the Liebermann-Storch reaction<sup>2</sup>. If the quantity of rosin acids is desired, it may be determined by the Twitchell method<sup>3</sup>.

## UNSULFONATED RESIDUE OF OIL.

## REAGENT.

*38 N sulfuric acid.*—Prepare as directed under turpentine<sup>4</sup>.

## DETERMINATION.

Weigh 5 grams of the recovered oil into a Babcock cream bottle. To this add slowly 20 cc. of the 38 N sulfuric acid. Mix the contents gradually, being careful that the temperature does not rise above 60°C. When the mixture no longer warms on shaking, agitate thoroughly, place in a water bath, and heat to 60°–65°C. for 10 minutes, keeping the contents of the flask thoroughly mixed by shaking vigorously five or six times. Fill the flask with strong sulfuric acid until the oil rises into the graduated neck. Centrifugalize 4–5 minutes at about 1200 revolutions per minute. Read the volume of unsulfonated residue from the graduations on the neck of the bottle, determine the

<sup>1</sup> *Mitt. Materialprüfungsamt*, 1918, 36: 279; C. A., 1919, 13: 3312.

<sup>2</sup> Lewkowitsch. *Chemical Technology and Analysis of Oils, Fats, and Waxes*, 5th ed., 1913, 1: 610.

<sup>3</sup> *Ibid.*, 625.

<sup>4</sup> *Methods of Analysis*, A. O. A. C., 1925, 408, 83.



TABLE  
Collaborative results—  
SAMPLE

ANALYST	WATER		OIL		FATTY AN- HYDRIDES REFEREE'S METHOD
	Referee's Method	California Method	Referee's Method	California Method	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
W. A. De Long	32.93	33.29	62.80	58.49	3.79
Macdonald College	32.56	33.10	....	58.37	3.85
Quebec, Can.					
Average	32.75	33.20	62.80	58.43	3.82
John W. Elmore	33.20	33.60	62.32	60.51	3.81
Department of Agriculture	33.28	33.80	62.21	60.78	3.84
Sacramento, Calif.					
Average	33.24	33.70	62.27	60.65	3.83
J. J. T. Graham	33.97	33.04	61.56	60.45	3.81
	33.85	33.22	61.63	60.84	3.85
Average	33.91	33.13	61.60	60.65	3.83
R. E. Andrew	33.22	....	....	61.43	....
Agricultural Experiment Station	33.28	....	....	62.07	....
New Haven, Conn.					
Average	33.25	....	....	61.75	....
General Average	33.29	33.34	62.10	60.37	3.83

SAMPLE

W. A. De Long	35.42	36.83	....	60.26	0.94
	35.82	37.05	63.29	60.38	0.94
Average	35.62	36.94	63.29	60.32	0.94
J. W. Elmore	36.80	37.60	61.73	60.39	1.21
	36.88	37.20	61.72	60.57	1.14
Average	36.84	37.40	61.73	60.48	1.18
J. J. T. Graham	37.27	36.23	61.41	60.70	1.15
	37.57	36.54	61.31	60.58	0.96
Average	37.42	36.39	61.36	60.64	1.06
R. E. Andrew	36.71	....	....	62.34	....
	36.93	....	....	61.64	....
Average	36.82	....	....	61.99	....
General Average	36.68	36.91	61.89	60.86	1.06

## 1.

*mineral oil-soap emulsions.*

## I.

SOAP			UNSULFONATED RESIDUE		SODIUM OXIDE— REFEREE'S METHOD	POTASSIUM OXIDE— REFEREE'S METHOD	ASH— CALIFORNIA METHOD
Calculated from fatty anhydrides —Referee's Method	California Method I	California Method II	Referee's Method	California Method			
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
4.44	4.56	4.26	57.83	58.40	....	0.64	....
4.51	4.41	4.43	57.65	58.36	....	0.62	....
4.48	4.49	4.35	57.74	58.38	....	0.63	....
4.46	4.59	4.86	59.20	57.80	....	0.67	1.09
4.50	4.52	4.89	59.70	57.80	....	0.67	1.08
4.48	4.56	4.88	59.45	57.80	....	0.67	1.09
4.46	4.59	4.72	57.71	56.50	....	0.66	1.06
4.51	4.59	4.77	....	....	....	0.67	1.03
4.49	4.59	4.75	57.71	56.50	....	0.67	1.05
....	4.31	....	....	....	....	0.63	1.04
....	4.45	....	....	....	....	0.65	1.08
....	4.38	....	....	....	....	0.64	1.06
4.48	4.50	4.66	58.42	57.77	....	0.65	1.06

1.05	1.43	1.25	83.39	85.52	0.15	..	....
1.05	1.43	1.22	83.40	85.48	0.15	....	..
1.05	1.43	1.24	83.40	85.50	0.15	....	....
1.35	1.41	1.30	86.50	84.90	0.26	..	0.29
1.27	1.47	1.33	86.50	85.20	0.26	.	0.29
1.31	1.44	1.32	86.50	85.05	0.26	.	0.29
1.28	1.48	1.42	85.44	85.06	0.15	...	0.28
1.07	1.48	1.47	....	....	0.16	..	0.29
1.18	1.48	1.45	85.44	85.06	0.16	..	0.29
....	1.38	....	....	....	0.14	..	0.28
....	1.48	....	....	....	0.15	.	0.29
....	1.43	....	....	....	0.15	....	0.29
1.18	1.45	1.33	85.05	85.23	0.18	....	0.29

density of the oil, and from these values calculate the weight and percentage of the unsulfonated oil.

#### ASH.

Evaporate 10 grams of the sample, or more if necessary, in a platinum dish; ignite to a white ash and weigh. From this weight calculate the percentage of ash in the sample. Test the ash for copper, calcium, calcium fluoride, etc.

### REFEREE'S METHODS FOR THE ANALYSIS OF MINERAL OIL-SOAP EMULSIONS.

#### WATER.

Weigh about 25 grams of the sample into a 300–500 cc. flask, add 50 cc. of xylene and, if necessary to prevent foaming, a small piece of rosin. Distil into a distilling tube receiver of the type described by Dean and Stark<sup>1</sup>. Continue the distillation until no more water collects in the receiver. Allow the contents of the tube to cool to room temperature, read the volume of water under the xylene in the tube, and from this volume calculate the percentage of water.

#### FATTY ANHYDRIDES.

Weigh about 20 grams of the sample into a Squibb separatory funnel, add about 40 cc. each of water and ether, and thoroughly mix by gentle shaking. If necessary, break the emulsion with alcohol, using as small a quantity as possible. Draw off the aqueous layer into a second funnel and wash it with ether. If an emulsion is formed in this washing, break it in the same manner as previously. Again draw off the aqueous layer into a third funnel and wash it with ether. Draw off the aqueous layer into a fourth funnel. Next successively wash the ether fractions with two portions of water. Combine these washings with the original aqueous layer, acidify with dilute sulfuric acid, and extract three times with ether. Wash the ether extracts from the acidified solution twice with water. Collect the washed extracts in a weighed beaker, evaporate on the steam bath, and weigh as fatty acids. Calculate the percentage of fatty anhydrides, using the factor 0.97.

#### SODIUM OR POTASSIUM OXIDE.

Weigh about 10 grams of the sample into an evaporating dish, heat on the steam bath until the water is expelled, and char the residue at a low temperature. Digest the charred mass with hot water; filter; wash thoroughly with hot water; and titrate the filtrate with 0.5 *N* sulfuric or hydrochloric acid, using methyl orange as indicator. From the number of cc. of the acid used, calculate the percentage of sodium or potassium oxide.

#### MINERAL OILS.

Determine mineral oils by difference.

#### UNSULFONATED RESIDUE OF OIL.

Evaporate off the solvent from the first set of ether extracts obtained under "Fatty Anhydrides", taking care to prevent loss of the oil, especially if it is a low-boiling fraction.

Determine the unsulfonated on this residue of oil as described on p. 129.

The collaborative results are given in Table 1.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 486.

## COMMENTS BY ANALYSTS.

*R. E. Andrew.*—California Method II for soap and the referee's method for fatty anhydrides both gave emulsions in the separatory funnels that were very difficult to handle, and the addition of alcohol did not facilitate the separation. No figures have been obtained by either of these two methods.

*W. A. De Long.*—For the determination of water the referee's method is preferred, chiefly on account of the advantage afforded of having the contents of the distilling flask constantly under observation.

In the California method kerosene was found to be much more effective than xylene in preventing foaming. It would be of some advantage to have stated the approximate amount of kerosene or xylene necessary to accomplish this purpose. A volume of 100 cc. per 50 grams of emulsion was found quite satisfactory with the samples analyzed.

In the California method for the determination of total oil it would appear desirable that a definite statement regarding the mode of determination of the density of the oil layer after centrifuging be incorporated. The same criticism would also apply to the similar determinations required in the estimation of the unsulfonated residue of the oil. Further, inasmuch as variations in the density cause considerable differences in the percentage of total oil or unsulfonated residue found, it would seem desirable to have this constant reported on in collaborative work.

In the determination of soap by Method II of the California procedure, it was found necessary to break the emulsion formed when extracting with 50 per cent alcohol, and on the advice of the referee sodium hydroxide solution was used for this purpose. Filtering of the precipitated soap was most conveniently done on a Büchner funnel, gentle suction being used. Solution of the soap precipitate in any convenient volume of hot water was found to be impracticable. A suggestion from the referee that the residue on the filter paper be transferred directly to a separatory funnel was followed. The presence of the filter paper was found to interfere somewhat in the subsequent extractions with ether, however, so that this means of disposing of the difficulty was not entirely satisfactory.

In the determination of fatty anhydrides charring of the residue was experienced at first. This trouble disappeared on more thorough washing of the ether extracts of the acidified solution. It was found necessary, however, to heat for about 40 minutes before the loss in weight of the fatty acids became small enough to neglect.

The criticism that the use of alcohol for breaking the emulsion tends to favor low results in this estimation is considered just, especially as it was found necessary to add considerable amounts of alcohol in order to accomplish that effect. This seemed particularly true in the case of the kerosene-soap emulsion.

*John W. Elmore.*—In the determination of ash, it was necessary to leach the charred residue in order to obtain a white ash.

In extracting the soap in California Method II, it was necessary to use some stronger alcohol to break the emulsion, after which the recommended strength was used. In the determination of the unsulfonated residue of Sample I, in both methods it was necessary to centrifugalize longer than the recommended time to obtain a constant reading.

## DISCUSSION.

The results for water by the two methods check fairly well. In the California method the liquid has a tendency to foam especially when xylene is used as a diluent. There is also objection to the use of a

copper distilling flask since it does not allow observation of the contents during the distillation. These objections are not met with in the xylene distillation method, and it is to be preferred to the California method.

For the determination of total oil the California method is more accurate than the referee's method. In the latter method the oil is determined by difference, and consequently the value is affected by the errors of all the other determinations.

Of the methods for fatty anhydrides and soap none is entirely satisfactory. In the referee's method the use of alcohol for breaking the emulsion is objectionable for the reason that it exerts a solvent action on the soap and carries part of it along with the ether solution of the oil, thus causing low results for fatty anhydrides.

California Method I is rapid and easy to carry out, but in the case of samples containing free alkali the results will be high, since all the alkali is titrated and calculated as soap.

In California Method II the precipitated soap is difficult to filter, and the residue apparently retains sufficient salt to prevent its solution in water for the extraction of the fatty acids.

The referee has modified this method by acidifying the soap solution, extracting and weighing the fatty acids instead of first precipitating the soap with a saturated sodium chloride solution. This modification gave very good results and was much easier to carry out than the original method.

In the determination of the unsulfonated residue of the separated oil, if the percentage was determined by volume instead of by weight the information gained would be equally as valuable, and the determination would be greatly simplified.

The method for ash should be changed to include leaching the residue from preliminary charring and subsequent complete ashing of the residue. Without leaching, it is very difficult to obtain a white ash.

#### SOAP.

##### PREPARATION OF SAMPLES.

Two samples of soap were prepared according to the following formulas:

##### *Soda Soap—Sample 3.*

Fish oil.....	3400 grams
Sodium hydroxide .....	500 grams
Water.....	2000 cc.

##### *Potash Soap—Sample 4.*

Fish oil.. .. .	3400 grams
Potassium hydroxide.....	700 grams
Water.....	2500 cc.

In each case the alkali was dissolved in about 1,500 cc. of water; the oil and the remainder of the water were poured into a steam-jacketed kettle, and after heating the alkali solution was added in small quantities with constant stirring. When saponification seemed complete the soap was transferred to a flat pan, allowed to cool, and was then packed in glass jars and sent with the following directions to the collaborating chemists.

#### METHODS FOR THE DETERMINATION OF WATER IN SOAP.

##### *Xylene Distillation Method.*

Weigh about 20 grams of the sample into a 300–500 cc. flask and add 50 cc. of xylene; in order to prevent foaming, add also about 10 grams of lump rosin. (Do not use powdered rosin, as it usually contains an appreciable quantity of moisture.) Distil into a distilling tube receiver of the type described by Dean and Stark<sup>1</sup>. Continue the distillation until no more water collects in the receiver. Allow the contents of the tube to cool to room temperature, read the volume of water under the xylene in the tube, and from this volume calculate the percentage of water.

##### *Official Method.*

Proceed as described in the official methods<sup>2</sup>.

The collaborative results are given in Table 2.

TABLE 2.  
*Determination of water in soaps.*

ANALYST	SAMPLE NO. 3		SAMPLE NO. 4	
	Official Method	Xylene Distillation Method	Official Method	Xylene Distillation Method
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
R. E. Andrew	30.00	29.95	32.06	32.66
	29.92	29.96	32.08	32.49
	Average	29.96	32.07	32.58
John W. Elmore	29.50	30.20	32.80	32.95
	29.70	30.10	32.90	32.60
	Average	29.60	32.85	32.78
W. A. De Long	28.46	29.24	34.54	33.40
	27.84	29.01	34.45	33.31
	Average	28.15	34.50	33.36
J. J. T. Graham	27.68	30.88	27.79	32.34
	27.24	30.63	27.63	32.40
	Average	27.46	27.71	32.37
General Average	28.79	30.00	31.78	32.77

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 486.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 65.

## DISCUSSION.

An examination of the results in Table 2 shows that the xylene distillation method for water gave much closer agreement among the analysts than the official method.

In the xylene method, in order to prevent foaming during the distillation, it is necessary to add about 10 grams of lump rosin. It is very important that no powdered rosin be used, since rosin in this form contains an appreciable quantity of water; lump rosin contains so little water that its effect on the result is negligible.

The only error introduced by lump rosin is in the case of soaps containing an excess of alkali. In such samples the excess alkali will react with the rosin to form rosin soap and water. However, the excess alkali in soaps will rarely be of such a quantity as to cause a very appreciable error in the results.

## SUGGESTIONS FOR FUTURE WORK.

The referee suggests that the work on mineral oil-soap emulsions be continued, the following methods being used:

## WATER.

Xylene distillation method, p. 132.

## TOTAL OIL.

California method, p. 128, except that the bottles are to be whirled 5 minutes instead of 2, as given in the method this year.

## SOAP.

California Method I; and California Method II modified as follows:

Weigh 20 grams of the sample into a separatory funnel, add 60 cc. of petroleum ether, and extract the mixture once with 20 cc. and four times with 10 cc. of 50 per cent alcohol. Break the emulsion if necessary with 1 or 2 cc. of a strong solution of sodium hydroxide, allowing the solution to run down the side of the separatory funnel, which is then gently twirled and allowed to stand for a few minutes. Draw off the alcoholic layers and wash them successively through petroleum ether contained in two other separatory funnels. Combine the alcoholic extracts in a beaker and evaporate on a steam bath to remove the alcohol. Dissolve the residue in about 100 cc. of water made alkaline with sodium hydroxide. Transfer to a separatory funnel, acidify with hydrochloric or sulfuric acid, extract three times with ether, and wash the ether extracts twice with water. Combine the ether extracts, evaporate in a tared beaker on a steam bath, and weigh as fatty acids. From the weight of fatty acids calculate the percentage of soap in the sample as sodium or potassium oleate.

## UNSULFONATED RESIDUE.

Modify the California method by substituting "measure 5 cc." for "weigh 5 grams" and changing the last sentence to read as follows: "Read the volume of unsulfonated residue from the graduations on the neck of the bottle and from this value calculate the percentage by volume of the unsulfonated oil".

## ASH.

Evaporate 10 grams of the sample, or more if necessary, in a platinum dish; ignite; and leach the charred mass with water. Ignite the residue, add the leachings, evaporate to dryness, ignite, and weigh. From this weight calculate the percentage of ash. Test the ash for copper, calcium, calcium fluoride, etc.

Another subject that should receive early consideration is a study of methods of analysis for cyanides. The association has at present methods for the determination of cyanogen and chlorine in sodium and potassium cyanides, but criticisms of these methods have been received from two firms handling the materials.

Calcium cyanide is also growing in importance as an insecticide and methods for its analysis should be studied.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the work on mineral oil-soap emulsions be continued, the methods suggested by the referee being used, p. 137, and that these methods be adopted as tentative methods with the view to their adoption as official methods after further cooperative study in 1926.

(2) That the xylene distillation method for water in soaps, p. 135, be adopted as a tentative method with the view to its adoption as an official method after further cooperative study in 1926.

(3) That the Kissling method for the determination of nicotine in tobacco and tobacco extract be dropped as an official method. (Second and final action.)

*A. G. Murray:* Mr. Chairman, I notice that considerable difficulty has been encountered in connection with the extraction of the fatty acids of the soaps by the usual separatory funnel procedure. It occurred to me that an automatic extraction device, such as has been used in the Drug Control Laboratory of the Bureau of Chemistry, might help. One of these extractors was exhibited here last year, and perhaps some of the members saw it. Some of our drug samples, as you know, give a great deal of trouble with emulsions. This year a sample of ipecac fluidextract, which ordinarily gives a great deal of trouble with the separatory funnel method, worked very well when the device was used.

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No formal report was given by the Referee on Soils and Liming Materials. The referee expressed approval of the reports of the associate referees and concurred in their recommendations for future work<sup>2</sup>.

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9 71.

<sup>2</sup> *Ibid.*, 73.



## REPORT ON REACTION VALUE OF SOILS.

By P. S. BURGESS (Agricultural Experiment Station, Tucson, Ariz.),  
*Associate Referee.*

The reaction values of soils are at present expressed in the conventional terms of pH units, which are the logarithmic exponents, or powers of ten, of the hydrogen-ion concentrations in terms of normality, with the sign changed to positive<sup>1</sup>. Hydrogen-ion concentrations may be determined by two general methods, (1) the electrometric method, requiring either a potentiometer or a millivoltmeter, the differences in potential due to H-ion concentrations being directly measured; (2) the colorimetric method, requiring standard buffer solutions of known and constant H-ion concentrations, proper indicators, and a comparator for matching the colors produced. (It is taken for granted that all are familiar with the principles and the general operation of the methods indicated, so that no detailed description is needed.) In soil work the former method is very largely used, for here turbidity and color changes in the solutions do not preclude exact results, but as the entire idea of H-ion concentration as applied to soils is comparatively new, no universally accepted method for its determination has as yet been agreed upon among soil scientists.

As no soil analysis on modern lines can be considered complete which does not include a determination of its reaction, and as different methods give slightly different results, it is obvious that, to render results comparable and intelligible to all, a uniform procedure in making this determination should soon be adopted.

As soil extracts, even after being centrifuged, seldom exhibit sufficient clarity and freedom from color (and this is especially true of alkaline soils) to permit of the general use of the colorimetric method, it was decided that all the preliminary work should be done with the more generally applicable electrometric method.

It was desired that the following questions be answered:

1. What is the proper or best proportion of water to soil to use?
2. What is the optimum length of time of extraction or shaking the suspension before the readings are taken?
3. What is the effect of drying soils on pH measurements?
4. What concentration of nitrates in soils will preclude the use of the electrometric method?
5. Do considerable quantities of electrolytes in soils (as in alkali soils) interfere with or modify the results secured?
6. Is the present method of reporting results (as pH units) satisfactory, or should a new system be devised?

<sup>1</sup> Sorensen, S. P. L., and Linderstrom—Lang, K. On the determination and value of  $\pi_0$  in electrometric measurements of hydrogen-ion concentrations. *Comp. rend. trav. lab. Carlsberg*, 1924, 15: 1.

The associate referee first thought of sending to collaborators some half-dozen samples of soils of different reaction, with directions as to proportions of water, time of shaking, etc., but after more mature thought it seemed to him that more real progress this first year would be made by reviewing the literature of the past few years and making a series of determinations on his own potentiometer. In this way a general idea concerning the points previously enumerated could be obtained and the recommendation made that, this coming year, with the information thus secured as a guide, collaborative work be undertaken.

The following is a brief summary of the work on the points in question, together with a list of references to recent literature which seems to have a direct bearing:

1. The best proportion of water to soil would seem to be 5 to 1. This gives results in fair accord with field observations, and at present it is the proportion generally used in soils laboratories. There is a slight but gradual decrease in acidity with dilution<sup>1</sup>.

2. Little difference was noted in results secured between shaking 15 minutes, 30 minutes, and 45 minutes, although there is a decided tendency for acid soils to become more acid on prolonged extraction, owing, probably, to slow hydrolysis of slightly soluble aluminium and iron salts. A shaking period of 30 minutes would seem to be sufficient. Intermittent shaking appears to be as good as constant agitation<sup>2</sup>.

3. The associate referee's work on the effect of air drying and oven drying soils on H-ion concentration would indicate that drying acid soils has but little effect on H-ion concentrations, although there appears to be a tendency toward slightly increased acidity at oven temperatures. With alkaline soils, drying renders them decidedly less alkaline, decreases of from 0.2–0.4 of a pH unit being general. Other investigators have reported similar results. It is recommended that fresh soils be used where possible, although air drying changes results but slightly. When air-dried soils are analyzed, it should be so stated in the paper reporting the work<sup>3</sup>.

<sup>1</sup> Atkins, W. R. G. The hydrogen-ion concentrations of some Indian soils and plant juices. Bull. 136, Agricultural Research Institute, Pusa, India, 1922; Crowther, E. M. Studies on soil reaction. III. The determination of the hydrogen-ion concentration of soil suspensions by means of the hydrogen electrode. *J. Agr. Sci.*, 1925, 15, part 2: 201; Hudig, J., and Sturm, W. The determination of H-ion concentration in soil extracts and soil suspensions. *C. A.*, 1919, 13: 2945; *Experiment Station Record*, 1920, 42: 313; Salter, R. M., and Morgan, M. F. Factors affecting soil reaction. I. The soil-water ratio. *J. Phys. Chem.*, 1923, 27: 117; Sharp, L. T., and Hoagland, D. R. Acidity and adsorption in soils as measured by the hydrogen electrode. *J. Agr. Research*, 1916, 7: 123.

<sup>2</sup> Crowther, E. M. See reference above; Joseph, A. F., et al. Annual Reports of the Government Chemist for 1921, 1922, 1923, and 1924. Welloome Tropical Research Laboratories, Khartoum, Soudan.; Salter, R. M., and Morgan, M. F. See reference above; Tidmore, J. W., and Parker, F. W. Methods of studying the strength of soil acids. *Soil Sci.*, 1924, 18: 331.

<sup>3</sup> Burgess, P. S. The hydrogen-ion concentration of soils as affected by drying. *Science*, 1922, 55: 647; Healy, D. J., and Karraker, P. E. The Clark hydrogen-electrode vessel and soil measurements. *Soil Sci.*, 1922, 13: 323; Hudig, J., and Sturm, W. See reference above; Plummer, J. K. Studies in soil reaction as indicated by the hydrogen electrode. *J. Agr. Research*, 1918, 12: 19.

4. Nitrates will not usually interfere with electrometric determinations up to 400 parts of  $\text{NO}_3$  per million of soil. This factor should be studied for a large number of soils of different genesis and reaction<sup>1</sup>.

5. Electrolytes (aside from the carbonates of alkalis and alkaline earth metals) do have an effect on pH determinations. The effect of sodium sulfate and sodium chloride is to increase acidity slightly, due doubtless to cation replacement and subsequent hydrolysis. This point should be studied more fully by soil investigators in the West where white alkali soils are of common occurrence<sup>2</sup>.

6. It is not considered that the present method of reporting results as pH units is the best that could be devised, although it is at the moment almost universally used. Any comparison of exponential values by direct observation is always puzzling. It must constantly be borne in mind that a pH of 7.0 indicates approximate neutrality (the pH of pure water), while a pH of less than 7.0 indicates an excess of H-ions (acidity) and a pH greater than 7.0 shows an excess of OH-ions (alkalinity). Take, for example, two soils where pH values are 5.2 and 5.8, respectively. It is known at a glance that both of these soils are quite acid, but it will require considerable calculation to show that the first is approximately four times as acid as the second. This, however, is the case. A few years ago E. T. Wherry, of the Bureau of Chemistry of the United States Department of Agriculture, proposed his "Specific Acidity and Specific Alkalinity Equivalents",<sup>3</sup> which show at a glance from H-ion measurements just how acid or alkaline a solution or soil may be in terms that are directly comparable. For instance, in the above example, the specific acidities of the two soils are 63 and 16, and it can be seen at once and with no laborious calculation that the first is four times as acid as the second. Wherry's table (somewhat abridged), whereby it is possible to transpose pH values directly to "specific acidity equivalents", follows:

pH.	SP. AC.	pH.	SP. AC.	pH.	SP. AC.	pH.	SP. AC.
4.5	315	5.3	50.	6.1	8.	6.9	1.30
.6	250	.4	40.	.2	6.3	7.0	1.00
.7	200	.5	31.5	.3	5.	.1	.80
.8	160	.6	25.	.4	4.	.2	.63
.9	125	.7	20.	.5	3.2	.3	.50
5.0	100	.8	16.	.6	2.5	.4	.40
.1	80	.9	12.5	.7	2.	.5	.32
.2	63	6.0	10.	.8	1.6	.6	.25

<sup>1</sup> Atkins, W. R. G. Some factors affecting the hydrogen-ion concentration of the soil and its relation to plant distribution. Roy. Dublin Soc. Sci. Proc., 1922, 16 (N. S.), Nos. 30-34: 369; Joseph, A. F., et al. See reference, p. 139.

<sup>2</sup> Atkins, W. R. G. See reference above. Clark, W. M. The determination of hydrogen-ions. Baltimore: Williams & Wilkins Co., 2d edition, 1922; Crowther, E. M. See reference, p. 139; Hudig, J., and Strum, W. See reference, p. 139; Joseph, A. F., et al. See reference, p. 139.

<sup>3</sup> The statement of acidity and alkalinity, with special reference to soils. *J. Wash. Acad. Sci.*, 1919, 9: 305; Note on specific acidity. *Ecology*, 1922, 3: 346; Wherry and Adams. Methods of stating acidity. *J. Wash. Acad. Sci.*, 1921, 11: 197.

RECOMMENDATION<sup>1</sup>.

It is recommended that this more concrete method of stating results, or some other equally as simple, be studied with a view to later adoption as a standard.

## REPORT ON LIMING MATERIALS.

(The Effect of Certain Impurities Upon Results by the Modified Scaife and Modified Sugar Methods.)

By W. M. SHAW (Agricultural Experiment Station, Knoxville, Tenn.),  
*Associate Referee.*

In the 1924 report of the associate referee<sup>2</sup>, a modification of the tentative sugar method for the determination of lime was offered. It was recommended that this method be studied further, with special reference to the effect of impurities. Since the modified Scaife method has been widely used, it was thought desirable to study the two methods in parallel. As a preliminary step the concordance of determinations by the two methods was established for high-grade calcic and magnesian limes.

## COMPARISON OF RESULTS BY THE MODIFIED SUGAR AND MODIFIED SCAIFE METHODS.

In this and subsequent experiments, samples were selected from the stock which had been sealed after completion of the 1924 collaborative studies. Table 1 gives the results of five determinations on each of four such samples by the modified sugar method, and Table 2 gives the 1924 results by the modified Scaife method. The results given in Table 1 show agreement within 0.1 per cent for the separate determinations by the modified sugar method for both the high-grade calcic and magnesian limes. The averages given in Table 1 are also in very good agreement with the grand averages of results by the modified Scaife method in Table 2.

## EFFECT OF CALCIUM ALUMINATE ADDITIONS.

Since it has been shown that aluminium oxide will readily react with calcium hydroxide in aqueous solution suspension<sup>3</sup>, the influence of the solubility of impurities of aluminium oxide in the calcined products was studied by adding ignited calcium aluminate in quantities giving 2, 5, and 10 per cent of the lime charges. The results are given in Table 3.

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 71.

<sup>2</sup> *This Journal*, 1925, 8: 344.

<sup>3</sup> *Soil Science*, 1925, 19: 125

From these results it appears that the additions of calcium aluminate exert distinctly different effects on the calcium oxide determinations by the two methods. The titration values of the lime-sugar solution are augmented in almost direct proportion to the addition of calcium aluminate, but in the modified Scaife method the 2, 5, and 10 per cent additions of calcium aluminate gave practically the same increase in the calcium oxide value of the samples. The experiment was repeated on another sample of high-grade calcium lime, with similar results.

Another experiment was set up to determine the differential behavior of calcium aluminate under the various conditions imposed by the two methods. Two quantities of calcium aluminate were suspended in water, in a 5 per cent sugar solution, in calcium chloride solution, and in a solution of calcium chloride plus calcium hydroxide. The calcium chloride concentrations, 1.0 per cent and 0.5 per cent, represented values equivalent to those obtained by the Scaife method from high- and low-grade limes, respectively. The calcium hydroxide addition was added to simulate the alkalinity of the lime solution under the average conditions of the same method. The results of these experiments are given in Table 4.

It is evident that the solubility of calcium aluminate is very appreciable in water and in 5 per cent aqueous sugar solutions. From a consideration of the relative quantities of lime and alumina it appears that all the lime and a part of the alumina had been dissolved by both the water and the sugar solutions. The figures given in Table 3 will show that the presence of calcium succrate had not greatly diminished the solvent action of the sugar solution upon the calcium aluminate. However, the presence of calcium chloride greatly depressed the solubility of the aluminate, its concentration in 1 per cent solution being only half of that found in either the water or the sugar solution. The data in Table 4 also show that a 0.01 *N* calcium hydroxide solution with a 1 per cent calcium chloride addition was sufficient to salt out completely the calcium aluminate. The same was also true for the 0.5 per cent calcium chloride addition. The increased titration values due to aluminate are small and may be within the limits of experimental error. The data of Table 4 thus corroborate and explain the differential effect of calcium aluminate on the calcium oxide value of the same lime when carried out by the two procedures here used, as shown in Table 3. It should be stated that since the calcium hydroxide concentration encountered in the Scaife method may be less than that used to obtain the results given in Table 4, it appears that there is a possibility of decreased solubility of calcium aluminate with an increased concentration of calcium hydroxide.

## EFFECT OF CALCIUM SILICATE.

Calcium silicate may be present as a result of reaction between silica impurities and calcium oxide during the process of calcination. Calcium silicate is also readily formed in aqueous suspensions of calcium hydroxide and silica. It was essential, therefore, to determine the effect of calcium silicate upon the procedures followed in the two methods. Calcium silicate was prepared by adding a solution of calcium chloride to one of sodium silicate. After washing and drying, the silicate was ignited. Two quantities, 0.070 and 0.140 gram, were shaken with 500 cc. of water and the following solutions: 5 per cent aqueous sugar solution, 0.5 and 1.0 per cent calcium chloride solutions and the same calcium chloride solutions plus sufficient calcium hydroxide to give an alkalinity of 0.095 *N*. The systems were shaken one hour and allowed to stand for 24 hours. Aliquots of 100 cc. of the clear solution were titrated with *N*/20 hydrochloric acid, and the alkalinities were expressed as percentage of the lime charge. The results are given in Table 5.

It is apparent that the solubilities of calcium silicate in water alone and in water containing 5 per cent of sugar are almost identical. The addition of 1.0 and 0.5 per cent calcium chloride considerably depressed the solubility of calcium silicate. The calcium hydroxide in the calcium chloride solution resulted in practically complete insolubility of the calcium silicate addition. Conversely the calcium silicate addition caused a diminution in the original calcium hydroxide concentration. The chemistry of this phenomenon is of sufficient importance to warrant further investigation. It is apparent that not only the concentration of calcium hydroxide but also the total concentration of Ca-ions is a factor in the reduction of alkalinity which was caused by the calcium silicate.

The experiment with calcium silicate was repeated with lime No. 8 (Tables 1 and 2), both the modified sugar and the modified Scaife methods being used. The usual time of contact, 20-30 minutes, was allowed. The results are given in Table 6.

It is evident that neither 5 nor 10 per cent additions of calcium silicate had any effect upon the calcium oxide value obtained by either of the two methods. These results are in apparent contradiction with those obtained from the addition of the calcium silicate to solutions of calcium chloride and calcium hydroxide, as shown in Table 5. The difference of time of contact may be responsible for this apparent discrepancy since, in the experiment with calcium hydroxide solutions, the contact period was 24 hours, while with the lime charges it was only 30 minutes. The lime solutions, therefore, were again titrated after 24 hours, and it was found that the solid phase calcium silicate had brought about a decreased concentration of calcium hydroxide, as in the previous experiment. This obtained with both the modified sugar and the Scaife

methods. The resultant decrease in the calcium oxide value was from 1.0–1.5 per cent of the charge. From the above results with calcium silicate it may be concluded that its presence in the lime sample has a tendency to produce a minus error by both the Scaife and the sugar methods. The magnitude of this error depends upon the period of contact. When contact extends for only 20–30 minutes the error attributable to calcium silicate is negligible.

The decided reduction in the calcium hydroxide concentration caused by the addition of the prepared calcium silicate casts some doubt as to its exact normal composition. Whether this phenomenon is caused by the absorption of calcium silicate or is a result of interaction of the calcium hydroxide with a slight excess of silicon oxide in the prepared product will be determined later.

#### SUMMARY.

Based on experiments with typical lime, it has been shown:

1. That the modified sugar method offers a procedure for rapid and concordant determinations of the caustic value of lime, as run parallel with the modified Scaife method in the hands of three collaborators.
2. That the presence of calcium aluminate in a lime will add proportionately to the calcium oxide value as shown by the sugar method, while the same substance will show only a slight effect on the calcium oxide value as given by the modified Scaife method.
3. That the presence of calcium silicate in a lime will cause no considerable minus error by either method, if the period of contact is not extended beyond one-half hour.

#### RECOMMENDATION.

It is recommended that the study of the modified Scaife and the modified Stone-Scheuch methods be continued.

TABLE 1.

*Concordance of individual calcium oxide determinations by the proposed modification of the sugar method\*.*

LIME NO.	1	2	3	4	5	AVERAGE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	40.2	40.1	40.2	40.1	40.0	40.1
4	68.3	68.2	68.2	68.0	67.8	68.1
6	94.3	94.2	94.1	94.3	94.5	94.3
8	91.5	91.8	91.8	91.5	91.5	91.6

\* Same limes as those of Table 2.

TABLE 2.

*Average of calcium oxide determinations secured by collaborators using the modified Scaife method\*.*

LIME NO	A-AVERAGE	B-AVERAGE	C-AVERAGE	GRAND AVERAGE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	39.8	40.1	40.2	40.0
4	68.3	68.6	68.3	68.4
6	94.3	94.4	93.9	94.1
8	91.2	91.9	91.5	91.5

\* Same limes as those of Table 1.

TABLE 3.

*Effect of added calcium aluminate on the calcium oxide determinations obtained by the modified Scaife and modified sugar methods.*

COMBINATION	MODIFIED SUGAR METHOD			MODIFIED SCAIFE METHOD		
	1	2	Average	1	2	Average
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Lime only . . . . .	91.8	91.5	91.7	91.6	91.8	91.7
Lime + 2 per cent calcium aluminate . . . . .	92.5	92.4	92.5	92.8	92.0	92.4
Lime + 5 per cent calcium aluminate . . . . .	...	93.8	93.8	92.7	92.5	92.6
Lime + 10 per cent calcium aluminate . . . . .	...	96.3	96.3	93.0	92.4	92.7

TABLE 4.

*Effect of certain concentrations of calcium hydroxide and calcium chloride upon the solubility of calcium aluminate, in terms of calcium oxide.*

CALCIUM ALUMINATE ADDED	IN WATER	IN 5 PER CENT SUGAR SOLUTION	IN CaCl <sub>2</sub> SOLUTION		IN 0.0095 N Ca(OH) <sub>2</sub> + *	
			0.5	1.0	CaCl <sub>2</sub> 0.5	CaCl <sub>2</sub> 1.0
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<i>gram</i>						
None	0.0	0.0	0.0	0.0	9.5	9.3
0.070	2.8	2.8	2.0	1.5	9.8	9.7
0.140	5.3	5.5	3.3	2.5	10.0	9.7

\* Simulating the usual alkalinity of the solution at the end of the Scaife method titration

TABLE 5.

*The solubility of calcium silicate in aqueous calcium chloride and calcium hydroxide—terms of calcium oxide per charge of lime.*

CaSiO <sub>3</sub>	IN H <sub>2</sub> O		IN 5 PER CENT SUGAR SOLUTION		IN 1 PER CENT CaCl <sub>2</sub>		IN 0.5 PER CENT CaCl <sub>2</sub>		IN 0.095 N Ca(OH) <sub>2</sub> +			
									1 per cent CaCl <sub>2</sub>		0.5 per cent CaCl <sub>2</sub>	
	<i>cc.*</i>	<i>per cent</i>	<i>cc.*</i>	<i>per cent</i>	<i>cc.*</i>	<i>per cent</i>	<i>cc.*</i>	<i>per cent</i>	<i>cc.*</i>	<i>per cent</i>	<i>cc.*</i>	<i>per cent</i>
<i>gram</i>												
None	...		...		...		...		18.7	9.4	19.0	9.5
0.070	0.8	0.4	0.8	.40	.6	.3	.6	.30	16.7	8.4	17.6	8.8
0.140	1.0	0.5	0.9	.45	.8	.4	.7	.35	15.4	7.7	16.6	8.3

\*0.05 N titer.



TABLE 6.

*Effect of calcium silicate additions on the determination of free calcium oxide in lime.*

COMBINATION	SUGAR METHOD			MODIFIED SCAIFE		
	1	2	Average	1	2	Average
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Lime only.....	91.8	91.5	91.7	91.6	91.8	91.7
Lime + 5 per cent CaSiO <sub>3</sub> .....	91.8	92.3	92.0	91.8	91.5	91.7
Lime + 10 per cent CaSiO <sub>3</sub> .....	91.3	91.5	91.4	91.5	91.5	91.5

## REPORT ON FEEDING STUFFS.

By L. E. BOPST (Assistant State Chemist of Maryland, College Park, Md.), *Referee*.

At the 1924 meeting of the association it was recommended that additional work be done on the direct determination of moisture. Wilbur Sterling was appointed associate referee on this subject, and during the current year he has conducted extensive collaborative work. His report, which will be given later, indicates clearly that this method has considerable merit. The general referee concurs in the recommendation that Sterling will make to the extent that this method be adopted by the association as tentative, but suggests that during the coming year additional work be performed, particular stress being put on its value as a rapid method for the determination of moisture and also for obtaining a correct moisture result on those products difficult to handle with the methods now in use.

M. R. Coe, the Associate Referee on Linseed Meal, has been actively engaged during the past year in carrying on collaborative work with the idea in mind of satisfying the association that the method as devised for the determination of starch in the presence of interfering polysaccharides is substantially accurate. His results indicate that it is now in such shape that the general referee feels justified in approving his recommendation that the method be finally adopted as official.

H. E. Gensler, the Associate Referee on Stock Feed Adulteration, who was not able to give a report last year, has been conducting investigational work along two distinct lines. First, the detection of salt in cattle feeds; second, an endeavor to devise some means of determining approximately the quantity of oat hulls in oats and oat feed mixtures.

In view of the excellent results obtained with the method devised for the determination of salt, and also in view of the fact that this method has already been adopted as a tentative one by the association, the referee suggests that Gensler's recommendation that this method be adopted as official be approved.

The results obtained in following the method devised by Gensler for the approximation of oat hulls in ground oats and oat mixed feeds have shown clearly that this method has considerable value. The referee is of the opinion, however, that this method could be improved considerably by more rigid wording, and therefore recommends that further work be done on this subject during the coming year.

A special problem relative to feeding stuffs was called to the referee's attention by the association's secretary in a communication received during the month of June, in which was enclosed correspondence from H. A. Halvorsen, Chemist in Charge of Feed Control, St. Paul, Minn. It was suggested that the association take up the investigation of a line of work that would have as its main object the formulation of methods which could be used in the examination of mineral mixture feeds. The general referee immediately wrote to Halvorsen requesting a brief digest of the methods in use in his laboratory and any other information pertinent to the subject that he would care to offer. This was done in order to avoid any repetition in the study of this subject elsewhere. It is regretted that this information was received too late for definite action this year. It is recommended, therefore, that an associate referee be appointed by the association to take care of this subject for the coming year<sup>1</sup>.

## REPORT ON THE METHOD FOR THE DETERMINATION OF STARCH IN THE PRESENCE OF INTERFERING POLYSACCHARIDES.

By MAYNE R. COE (Bureau of Chemistry, Washington, D. C.), *Associate Referee on Linseed Meal*.

With the assistance of the following collaborators the associate referee has continued the work on the method for the determination of starch in the presence of interfering polysaccharides as recommended:

- L. C. Mitchell, Food and Drug Inspection Station, St. Louis, Mo.;
- H. E. Gensler, Department of Agriculture, Harrisburg, Pa.;
- J. H. Mitchell, Clemson College, S. C.;
- V. R. Boswell, University of Maryland, College Park, Md.;
- J. I. Palmore, Bureau of Chemistry, Washington, D. C.;
- M. Jongeward, North Dakota Regulatory Department, Bismarck, N. D.

Three samples were prepared and sent out for study. The one to which starch was added, "A", represents a pure linseed meal entirely free from foreign material. The other two samples, "B" and "C", are typical of commercial linseed meal. "B" contained 8.2 per cent weed seeds, while "C" contained 3.8 per cent.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 71.

The results obtained with these samples show, in general, the adaptability of this method to material such as linseed meal. It is noticeable, however, that the figures for Sample A are not quite so close to the theoretical figure as would be expected, but the discrepancy can be accounted for by an uneven distribution of the added starch. All possible care was taken to produce a uniform sample, as it is well known how difficult it is to prepare such a sample so that the starch grains will not segregate. Taking that feature of the work into account, the figures given may be considered good.

The results found when the other two samples were used are noticeably closer and show a creditable application of the method for determining the adulteration of linseed cake with weed seeds. It may be stated also that the changes in the original method approved last year have made a decided improvement both in shortening the procedure and in manipulation.

*Results of Collaboration.*

	SAMPLE A	SAMPLE B	SAMPLE C
	4.2	2.3	1.9
	5.2	2.2	1.6
	3.4	1.7	1.4
	5.1	2.0	1.6
	3.5	1.8	1.5
	4.5	2.3	2.0
Average	4.3	2.1	1.5
Theoretical	4.2	..	..

Aside from the purpose of the method as mentioned, Collaborator Boswell, assistant in horticulture at the University of Maryland, has used it successfully for all leafy and storage tissue analysis. Another collaborator, J. I. Palmore, has used it with entire satisfaction in the analysis of mustards. In addition, the referee has received statements from others who say the method is clear and not difficult to follow.

It is recommended<sup>1</sup> that the method be adopted as official (final action).

## REPORT ON STOCK FEED ADULTERATION.

By H. E. GENSLER (State Department of Agriculture, Harrisburg, Pa.),  
*Associate Referee.*

As the result of a request made to the referee during the year that some means of determining approximately the amount of oat hulls in ground oats be suggested, an attempt was made to separate the hulls so that they could be weighed and later presented as visible evidence

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 72.

in court, if required. It was found that oat hulls, even though finely ground, show a marked tendency to collect in the bottom of a beaker of hot water while the bran and hairs float on the surface of the liquid. Although this separation is not always clean cut, the desired end can be obtained by careful manipulation. Simple hydrolysis of the starch by boiling the material in water slightly acidulated with hydrochloric acid was used as a means of dissolving the starch so that it could be decanted with the lighter particles.

Applying these principles to a study of ground oats, the referee boiled a portion with acidulated water. After pouring off the supernatant liquid with its floating particles, there remained a residue of hulls which proved under microscopic examination to be practically free from starch and the cells occurring in the hull. As subsequent determinations, in which various samples were used, indicated that it was possible to approximate the amount of hulls present, a description of the method was forwarded to the analyst in response to the request. It was later reported that the inquirer had obtained very satisfactory results, had gone into court, and had won the case. He also presented the following figures to show how the hull content, as determined by the method, tallied with the fiber.

	HULLS per cent	FIBER per cent
1 . . . . .	27.57	9.49
2. . . . .	28.01	10.04
3 . . . . .	30.14	10.92
4. . . . .	38.04	13.42
5 . . . . .	47.85	16.81
6 . . . . .	51.10	18.70

It will be noted that increase in hull content is more clearly shown by expressing it in terms of hulls than in terms of fiber—that is, the difference between No. 1 and No. 3 by hulls is 2.57 per cent, by fiber only 1.43 per cent; similarly between Nos. 1 and 6, the differences are 23.53 per cent and 9.21 per cent. An expression of the hull content, therefore, shows a graduation over the larger range.

In view of the results obtained, the referee decided that the method was worthy of a trial by the collaborators and accordingly presented it as follows:

#### DETERMINATION OF OAT HULLS IN GROUND OATS.

Place 2 grams of the sample, previously ground to pass through a sieve having circular openings 1 mm. in diameter, 150 cc. of water, and 5 drops of concentrated hydrochloric acid in a 300 cc. beaker and heat, stirring the mixture constantly, until it has boiled 2 minutes. Stir vigorously to obtain a centrifugal effect, allow to stand 15 minutes and then decant the supernatant liquid with sufficient care to permit only the very light particles to pass out. Add 100 cc. of water and heat to the boiling point while

stirring constantly. After stirring to centrifugalize the solid material, allow to stand until the supernatant liquid is quite clear. If some of the material floats on the surface, immediately add a small quantity of alcohol or ether in such a manner that it will form a surface above the water.

Draw off the supernatant liquid by means of a siphon of rubber tubing having a 3 or 4 mm. bore, using a pinch clamp to control the rapidity of flow and to prevent most of the liquid being drawn away. Tilting the breaker will also aid in procuring this result. If, after standing, the supernatant liquid is not clear, repeat the last step.

Transfer the hulls into a filter of smooth paper with the aid of hot water, followed by several washings of alcohol. Allow to dry to constant weight at room temperature. When dry carefully remove the hulls from the paper, using a small stiff brush if necessary.

$$\frac{\text{Weight obtained}}{n} \times 100 = \text{percentage of oat hulls.}$$

Samples of oats and oats containing added hulls upon which determinations were to be made were also submitted. Sample No. 1 was oats having approximately 30 per cent of hulls and represented an average oat according to figures obtained by investigators making a study of the amount of hulls in oats. Sample No. 2 was the same as No. 1, but ground. Sample No. 3 consisted of 95 per cent of Sample No. 2 and 5 per cent of ground oats. Sample No. 4 contained 85 per cent of Sample No. 2 and 15 per cent of oat hulls.

The collaborators were instructed to estimate the hulls in Sample No. 1 "by hand". The method submitted was to be used in analyzing the other samples.

Eighteen collaborators took part in this work, and the referee is greatly impressed by their interest and the accuracy shown. It is considered that the technique of this method is rather difficult for close checks, notwithstanding individuals reported the following results: 32.65 per cent, 32.54 per cent, and 32.18 per cent; 40.21 per cent, 40.21 per cent, and 40.14 per cent. The year's activity was well worth the effort, if for no other reason than to acquaint the referee with the splendid work being done in this field. The tabulation of the reports is shown in Table 1.

TABLE 1.  
*Oat hulls in oats.*

ANALYST	WHOLE OATS SAMPLE NO. 1 HULLS—29.80 PER CENT	GROUND OATS SAMPLE NO. 2 HULLS—29.80 PER CENT	GROUND OATS SAMPLE NO. 3 HULLS—33.31 PER CENT	GROUND OATS SAMPLE NO. 4 HULLS—40.33 PER CENT
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	28.60	30.78	35.15	38.80
2	29.40	31.88	34.69	41.43
3	29.15	30.04	33.00	39.08
4	30.09	29.56	33.12	37.20
5	31.30	31.60	35.70	40.00
6	28.25	32.39	35.35	40.88
7	28.60	32.60	35.10	38.30
8	28.55	32.43	35.39	40.09
9	28.00	30.00	....	40.00
10	31.80	31.11	34.26	40.75
11	29.75	31.59	34.89	40.00
12	28.87	33.49	35.88	40.61
13	29.45	32.46	36.42	40.19
14	27.44	35.30	37.50	42.53
15	27.65	32.68	34.38	37.92
16	27.23	31.86	35.60	41.10
17	27.63	32.67	34.95	40.44
18	28.50	34.00	36.30	42.10
Average	28.90	32.02	35.16	40.08

The figures for Sample No. 1 compared with those for Sample No. 2 indicate that it was about as difficult to determine the hulls in the unground sample as it was by using the method in the ground sample; the results on the unground sample range from 27.23–31.80 per cent and on the ground from 29.56–35.30 per cent. It is noticeable that the method gave results which were higher than the standard in the case of Samples Nos. 2 and 3. However, an analysis of the figures shows that the average of the results obtained on No. 2 is only 2.22 per cent above the standard (29.80 per cent). The average of No. 3 is 1.85 per cent above its standard (33.31 per cent). The average for No. 4, as well as the individual results, is quite close to the standard (40.33 per cent).

As to the technique, it is believed, as several collaborators suggested, that the instructions could be more definite and that some improvement could be made in the method itself. It would not be amiss to give the method further study because it would be impossible to make a separation of oat hulls in a sample after it has been ground, except to pick out the larger particles. The presence of ground screenings will always complicate any procedure involving separation of another component of a sample.

Considering this report from the standpoint of its practical application, it appears that the method will enable the microanalyst to present a fairly accurate estimate of the amount of hulls occurring in ground

oats and submit the hulls in verification of his figures. It should be understood that this method, as well as other similar methods, has its limitations and is not intended to supplant determinations for other constants, such as fiber. It is excellent laboratory procedure, however, to follow an analysis for fiber that is found to be above the average with an actual isolation and estimation of the material responsible for the rise in fiber.

A high fiber or hull content does not necessarily indicate that the oats have been adulterated. While oats average about 30 per cent of hulls they may normally contain more or less than this amount. H. B. Musser, of the Pennsylvania State College, in an examination of 600 samples, found that the hull content varied from 37 per cent to 25 per cent. Henry<sup>1</sup> reported that oats contain as high as 45 per cent of hulls. Hulls, other than those of oats, will also influence figures representing fiber or hull content. A microscopic examination will determine this point.

In interpreting results, therefore, consideration of these various factors must not be overlooked.

#### DETECTION OF SALT.

In addition to the samples just mentioned, the referee submitted Samples Nos. 5 and 6 for the detection of salt by his method as published previously<sup>2</sup>. Sample No. 5 was a ground mash feed and No. 6 a ground molasses feed. Each of the samples contained about one-half of one per cent of salt.

Table 2 shows the results obtained.

TABLE 2.  
*Detection of salt.*

ANALYST	SAMPLE NO. 5 SALT— $\frac{1}{2}$ OF 1 PER CENT	SAMPLE NO. 6 SALT— $\frac{1}{2}$ OF 1 PER CENT
1	Salt Present	Salt Present
2	" "	" "
3	" "	" "
4	" "	" "
5	" "	" "
6	" "	" "
7	" "	" "
8	" "	No Salt
9	" "	Salt Present
10	" "	" "
11	" "	" "
12	" "	" "
13	" "	" "
14	" "	" "
15	.....	No Salt
16	Salt Present	Salt Present
17	" "	" "
18	" "	" "

<sup>1</sup> Henry and Morrison. *Feeds and Feeding*, 1923, p. 162.

<sup>2</sup> *This Journal*, 1924, 7: 342; 1926, 9: 32; *J. Ind. Eng. Chem.*, 1923, 15: 158.

It will be seen that the method is reliable for determining whether or not salt has been added to a feed as distinguished from salt or chlorides normally present in the ingredients. This year's work shows a repetition of the favorable results obtained on samples previously presented.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That the method for the detection of salt be adopted as tentative.
- (2) That further study be made of the method presented for the determination of oat hulls in ground oats.

### REPORT ON METHOD FOR DETERMINATION OF MOISTURE BY DISTILLATION WITH TOLUENE.

By W. F. STERLING (Bureau of Chemistry, Washington, D. C.),

*Associate Referee.*

Composite samples of wheat shorts, meat meal, molasses feed, and cottonseed meal were ground to pass a 20-mesh sieve, thoroughly mixed, and placed in air-tight containers. Sets of these samples were sent to the collaborators, and the associate referee retained a set for moisture analysis.

#### INSTRUCTIONS TO COLLABORATORS.

A reprint of the paper, "Preliminary Notes on the Direct Determination of Moisture"<sup>2</sup>, presented at the meeting last year was also sent to the collaborators. It was requested that the samples be analyzed by this method (Method 1 in the table) and by the drying method is use (No. 2).

#### RESULTS.

Only three of the six collaborators submitted results. They are as follows:

COLLABORATORS	WHEAT SHORTS		MEAT MEAL		MOLASSES FEED		COTTONSEED MEAL	
	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2	Method 1	Method 2
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	7.52	7.91	5.76	5.90	5.78	6.33	5.94	5.99
2	9.35	9.28	6.58	6.80	7.79	7.63	7.11	7.17
3	8.49	9.13	6.77	6.32	7.60	7.96	6.49	6.65
*	8.90	8.72	6.55	6.56	7.43	7.43	6.68	6.69
Averages	8.57	8.76	6.42	6.39	7.13	7.34	6.56	6.63

\* Results obtained by the associate referee.

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9, 72

<sup>2</sup> *This Journal*, 1925, 8: 295.



RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the proposed distillation method for the determination of moisture in cattle feeds be adopted as tentative.

(2) That the method be studied further with a view to determining its value as a rapid procedure and its adaptability to materials that do not give reliable results by drying methods.

## REPORT ON SUGARS AND SUGAR PRODUCTS.

By H. S. PAINE (Bureau of Chemistry, Washington, D. C.), *Referee*.

The referee concurs in the recommendations embodied in the reports of the associate referees and recommends that work be continued along the lines which have been suggested.

The following additional comments are made with reference to Recommendations 3 and 5 of the Associate Referee on Polariscopic Methods (p. 177). The maximum concentration of raffinose in the mixtures of sucrose and raffinose that were used for analysis represents about the maximum raffinose concentration which is found in beet products after making the necessary dilution for analysis. Since the application of an analytical method for simultaneous determination of sucrose and raffinose is found practically exclusively in the analysis of beet products, it is recommended that the two-enzyme (invertase-melibiose) method be adopted as official.

The referee concurs in Recommendation 5 of the Associate Referee on Polariscopic Methods, with the understanding that extension of the investigation with quantities of raffinose greater than the maximum of 3 per cent used in this year's investigation is advisable from the standpoint of supplying data for analysis of unusual mixtures of sucrose and raffinose, rather than of supplying further data required in the analysis of beet products. It is recommended that the acid hydrolysis method for the determination of sucrose and raffinose<sup>2</sup>, which is now official, be made a tentative method, since its accuracy is greatly inferior to that of the invertase-melibiose method.

Reports have been received from all associate referees except the Associate Referee on Honey. William Seaman, who was appointed, resigned early in the year and efforts to fill this vacancy were unsuccessful.

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No report on honey was presented as no associate referee was appointed.

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 72.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 187.

## REPORT ON MAPLE PRODUCTS<sup>1</sup>.

By H. M. LANCASTER (Department of Health, Ottawa, Can.), *Associate Referee*.

As it appears that little or no advancement is made through collaborative work in which a number of analysts are furnished with different portions of the same sample for the testing out of a method of analysis, it has been considered advisable to concentrate upon certain problems of the maple industry. The following questions were proposed for investigation by experienced and competent analysts:

- (1) What is the general quality of maple sugars as supplied to manufacturing plants engaged in reprocessing sugar or in the reconstruction of maple sirup therefrom?
- (2) What changes occur in maple sugar and maple sirup when reprocessed in commercial plants?
- (3) Is there any method for the identification of cane sugar whereby the presence of the cane sugar product can be detected in admixture with maple products?

### INVESTIGATION OF MAPLE SUGARS AS SUPPLIED TO SO-CALLED MANUFACTURERS OF MAPLE PRODUCTS.

About 350 samples of commercial sugars have been examined, but the work is not yet completed, there being about 200 samples still unanalyzed. Results thus far indicate that comparatively little adulteration is practised by the small producer. Not more than 2 per cent of the sugars delivered to manufacturers shows any indication of the possibility of cane sugar having been added, although color and flavor might be greatly improved if the individual sugar maker followed a proper technique. Judging from the results thus far obtained, a genuine maple sugar from the Province of Quebec should carry a Canadian lead number of at least 2.5.

### INVESTIGATION OF COMMERCIAL REPROCESSING.

Several batches of maple sirup were sampled at different stages in treatment as practised by three different commercial firms operating on a large scale. Filtering through bags and filter pressing after admixture with kieselguhr have little or no effect upon the lead number. Prolonged boiling did appear to lower the lead number slightly in some sirups. Before making a definite pronouncement, it is considered advisable to include the results obtained in the reprocessing of sugars known to have been made during the 1925 season. The final samples have been collected at the factory, but the analytical work is not yet finished.

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<sup>1</sup> Presented by H. S. Paine.

## DETECTION OF CANE SUGAR IN MAPLE PRODUCTS.

An entirely new line of investigation has been started in the microscopical examination of the constituents of commercial cane sugar products, but the work has not advanced to a stage where a definite pronouncement can be made.

RECOMMENDATION<sup>1</sup>.

Practically the entire outline of work given in this report was in connection with commercial factories which were unexpectedly shut down for several months during the summer season. It is recommended that these studies be continued and completed.

No formal report was given on starch conversion products. The associate referee, F. W. Reynolds, stated that sufficient progress had not been made to warrant a report. It was recommended that the work be continued, as at present it appeared that the enzyme method was the only reliable method for the determination of maltose<sup>1</sup>.

## REPORT ON DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS.

By MAX SCHNELLER (38 Cranberry St., Brooklyn, N. Y.), *Associate Referee*.

Last year not less than seven modifications of drying methods for moisture determination in sugar-house products were the subject of collaborative analyses<sup>2</sup>, but for the present year J. F. Brewster, the former referee, recommended a reduction of this number. It is not an easy task to decide in favor of a few methods and eliminate others for insufficient reasons, while it must be admitted that working on samples of unknown moisture content, where the only criterion for judging results lies in more or less close agreement between the different methods and analysts, is practically groping in the dark. At the same time, it is evident that nearly all these methods suffer from the same faults, and that agreement of results is due merely to a compensation of a number of errors such as the following:

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 72.

<sup>2</sup> *This Journal*, 1925, 8: 375.

- (1) Persistent moisture retention by film formation and high vapor tension of concentrated sirups;
- (2) Oxidation;
- (3) Inversion of sucrose; and
- (4) Decomposition of invert sugar, especially fructose, resulting in the formation of secondary condensation products—or even caramelization and splitting off of water and volatile products.

A method may be accepted as satisfactory if it properly balances these divergent sources of error, and this condition can be true only in a limited field of application. Collaborative work has been confined to sugars and sugar-house products. In the lower grades of molasses, with their increasing invert sugar content, all methods become rather unsatisfactory, but their relatively high ash content, acting as a buffer, protects them from further inversion on drying, while confections and sirups prepared from refined sugar and partially inverted and frequently acidulated present the further difficulty of additional inversion at the drying temperatures used. This action must be prevented by previous careful neutralization with a measured quantity of alkali or buffer mixture. Most difficult is the moisture determination in invert sugar sirups marketed for the use of the confectionery and baking industries. The present referee, having been connected with the practical manufacture of such completely or semi-inverted products, offers in this report his efforts, though far from successful, toward a more accurate estimation of their true moisture content.

While mere probability, established by closest agreement among the largest number of collaborators, decided the value of a method in the case of sugar-house products, an important step toward application of more critical methods was made last year by including a synthetic sirup in the collaborative work. The criticism is made that this mixture was too complicated. It also is the opinion of the associate referee that greater certainty must first be established in moisture and density determinations of the simple and pure constituents before progress can be made critically with such complicated mixtures. Fructose, which introduces the most serious difficulties into drying methods, has heretofore been difficult to obtain, but through the efforts of R. F. Jackson of the U. S. Bureau of Standards it is now accessible in pure and non-hygroscopic crystalline form. Unfortunately, however, the small supply that the associate referee had was exhausted before the work was completed, and he was obliged to confine himself to invert sugar.

Reliable densimetric or refractometric tables are not available for glucose, fructose, or invert sugar. According to Stolle<sup>1</sup> the initial densities and refractive indices of freshly prepared solutions are, like their rotations, inconstant. A fragmentary invert sugar table containing values

<sup>1</sup> U. S. Bur. Standards Circ. 44.

by Chancel, Burkhard, and Herzfeld in v. Lippmann's handbook<sup>1</sup> is too incomplete and of insufficiently established value to serve for practical control work and still less as a guidance for moisture determinations by drying. Gerlach's sucrose table<sup>2</sup>, therefore, was employed in the routine practice described in this report—as everywhere in the sugar industry—but its shortcoming, naturally, is more apparent in a product that contains only a small portion, if any, sucrose. The readings, as is usual in sugar-house practice, were made in 1 : 1 dilutions, although with pure sugar sirup of low viscosity direct spindling might be feasible, and in fact it is regularly used in refinery practice for barrel sirup of 78° Brix. Precision hydrometers with a range of ten degrees checked with pure sucrose solutions were used. When later the Zeiss sugar refractometer was introduced into the routine work an average difference of 1.0° between hydrometer and refractometer readings was regularly observed.

It is the general opinion, supported by the observations of Tolman and Smith quoted in Browne's handbook<sup>3</sup> that a close agreement exists between the refractive indices of sucrose and most other soluble carbohydrates. Since the associate referee was able to confirm these findings in the case of 80 per cent commercial glucose coinciding exactly with that of sucrose, as much had been expected for invert sugar. But Tolman and Smith's tables do not include either invert sugar or dextrose.

Examples of these comparative densimetric and refractometric readings on completely and semi-inverted sirups are given in Table 1.

TABLE 1.  
*Comparative densimetric and refractometric readings.*  
COMPLETELY INVERTED SIRUPS.

° BRIX IN 1 : 1 DILUTION	REFRACTOMETER	DIFFERENCE	SUCROSE
			<i>per cent</i>
78.8	77.66	1.14	Less than 2
78.68	77.76	0.92	
78.86	77.76	1.10	
79.22	78.11	1.11	
76.84	75.61	1.23	
78.94	78.01	0.93	
SEMI-INVERTED SIRUPS.			
78.10	77.49	0.61	31.2
77.94	77.43	0.51	32.6
74.66	74.18	0.48	36.0
77.88	77.48	0.40	37.1
77.94	77.33	0.61	34.5
68.72	67.91	0.81	27.3

<sup>1</sup> *Chemie der Zuckerarten*, 1914, p. 917.

<sup>2</sup> *Z. Ver. deut. Zuckerind.*, 1901, 51: 335, 469.

<sup>3</sup> *Handbook of Sugar Analysis*, 1912, p. 62.

The fact that the deviation between Brix and refractometer degrees is in proper proportion—about half in the semi-inverted sirups—strengthened the conclusion that a lower refractive index of invert sugar is the cause of the observed differences.

Of the constituents of invert sugar, glucose only was available in sufficient purity. A 50 per cent solution was prepared from purest glucose purchased from the Bureau of Standards. Polarization of the normal weight of 32,264 grams of this solution in 100 cc. gave a constant reading of 49°V., and application of a correction for concentration established the correct concentration at 49.55 per cent glucose.

Refractometer readings for this solution, 49.47° and 49.4°, therefore, offered no explanation of the irregularities observed with invert sugar. The available supply of pure glucose was limited, and comparative densimetric measurements were omitted.

The simplest procedure to verify the indicated low refractometer results seemed to consist in preparing invert sugar solutions of definite concentration by careful inversion of sucrose sirups of known concentration with minimal quantities of hydrochloric acid. The exact procedure was as follows:

Twenty grams of purest sucrose (standard granulated sugar) was weighed on an analytical or sugar balance into small rubber-stoppered Erlenmeyer flasks, and 6, 8, 10, etc. up to 20 grams of distilled water was added. The sugar was dissolved in a boiling water bath; after cooling it was adjusted on the balance to the exact weights of 26, 28, 30, etc. up to 40 grams, and yielded eight sirups ranging from 76.92 down to 50.0 per cent sucrose. As a check these percentages were read on the refractometer to establish the correct adjustment of the instrument.

In another identical series extremely small quantities of hydrochloric acid (less than 0.01 per cent concentrated acid) were added to these sirups, and heating in the boiling bath was continued for about one hour or long enough to bring about an almost complete inversion. The resulting invert sugar solutions, perfectly colorless, were cooled and readjusted on the balance to their exact respective weights. The refractometer readings on these inverted sirups as well as the direct control readings are given in Table 2.

TABLE 2.

*Refractometer measurements on invert sugar solutions.*

SUCROSE	20 GRAMS SUCROSE BY REFRACTOMETER	SUCROSE AFTER INVERSION	TOTAL SOLIDS AFTER INVERSION— CALCULATED	REFRACTOMETER AT 20°	DIFFERENCE
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
76.92	76.90	1.54	80.69	78.77	1.92
71.43	71.65	1.54	74.92	73.12	1.80
66.67	66.75	1.87	69.91	68.37	1.54
62.50	62.55	1.87	65.53	64.12	1.41
58.83	58.85	1.65	61.69	60.27	1.42
55.56	55.60	1.54	58.26	57.12	1.14
52.63	52.65	1.54	55.18	54.17	1.01
50.00	49.99	1.54	52.42	51.37	1.05

The calculated total solids in this table were obtained by increasing the percentage of inverted sucrose by 5 per cent and adding to this figure the non-inverted sucrose ascertained analytically by the invertase modification of the Clerget method. The differences between the calculated and refractometer solids entirely confirm the previous observation.

This work was now repeated with a larger quantity of sugar, 150 grams of standard granulated, in order to permit a direct comparison with Brix hydrometer results in 1 : 1 dilution. The results are given in Table 3.

TABLE 3.

*Comparison of refractometer with hydrometer measurements on invert sugar sirup.*

SUCROSE AFTER INVERSION	TOTAL SOLIDS AFTER INVERSION	REFRACTOMETER AT 20° ON ORIGINAL	1 : 1 DILUTION		TOTAL SOLIDS MINUS REFRACTOMETER	TOTAL SOLIDS MINUS BRIX HYDROMETER	HYDROMETER MINUS REFRACTOMETER ON ORIGINAL
			Refractometer	Hydrometer			
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.99	80.69	78.85	79.4	80.04	1.84	0.65	1.19
1.10	80.69	78.85	79.4	80.24	1.84	0.45	1.39
1.54	74.92	73.3	74.0	74.64	1.62	0.28	1.34
1.65	69.91	68.5	68.7	69.68	1.41	0.23	1.18
1.76	65.53	64.2	64.4	65.46	1.33	0.07	1.26

From these figures it must be concluded that not only the sugar refractometer, but also the Brix hydrometer, gives too low results with invert sugar solutions. This conclusion agrees with the results of N. Schoorl<sup>1</sup> who determined the volume contraction occurring upon inversion and therefrom calculates that the Brix hydrometer readings must be increased by two-thirds of one per cent for application to invert sugar solutions. For an 80 per cent sirup this amounts to 0.54 per cent, and this figure

<sup>1</sup> Z. Nahr. Genussm., 1920, 39: 114.

is in close agreement with the average difference between "total solids minus Brix" for the 80 per cent sirup, 0.65 and 0.45. Due to probable inaccuracy this difference is evidently too low for the other sirups of less concentration and the difference "Brix minus refractometer" too high.

An objection might be raised against the method of inversion in the preceding procedure. While sirups thus prepared are quite colorless and show no apparent signs of decomposition, their polarization is abnormally low. A fully inverted sirup of 75° refractometer solids shows a constant polarization of only  $-20^{\circ}\text{V}$ . instead of  $-24^{\circ}\text{V}$ . at  $20^{\circ}\text{C}$ . By the action of hydrochloric acid in excess as in the Clerget analytical procedure at  $60^{\circ}$  or  $70^{\circ}\text{C}$ ., or at room temperature overnight, the normal polarization is approximately restored unless decomposition has taken place in the sirup, which is usually indicated by a yellow color. Invertase, on the other hand, causes no change of polarization in the case of a fully inverted sirup, the direct and invert reading remaining identical after 24 hours. Since in ordinary analytical practice the acid Clerget method is used, about 2.3 per cent sucrose is reported even for fully inverted sirup, and the Jackson and Gillis<sup>1</sup> method cannot reduce this error below 1.8 per cent.

This abnormal behavior of invert sirups is undoubtedly due to the presence of secondary condensation or reversion products formed in the presence of only minute quantities of acids. Such dextrin-like constituents, if present in sufficiently large quantities, might affect the total solids of a sirup to such an extent that the increase upon inversion would appear as less than 5 per cent over the sucrose employed.

If the total solids of invert sirup are thus influenced, the gain by sucrose inversion as measured with the refractometer in Tables 1-3 would be apt to vary according to the mode and time of heating, concentration of solutions, etc., while it would be constant if affected only by the deviation of the refractive indices of the sugars. A larger number of inversions as previously described were made, therefore, and the apparent hydration as read by the refractometer was recorded as percentage of the normal amount of hydration set at 5 per cent.

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<sup>1</sup> U. S. Bur. Standards Scientific Paper 375.



TABLE 4.

*Apparent hydration of sucrose during inversion.*

SAMPLE NO.	SUCROSE BEFORE INVERSION	SUCROSE AFTER INVERSION	CALCULATED SOLIDS	REFRACTOMETER AT 20°	APPARENT HYDRATION
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	76.92	0.00	80.77	78.45	39.5
2	71.43	0.22	74.99	72.9	41.3
3	66.67	0.44	69.98	68.3	48.9
4	62.50	0.88	65.58	64.0	48.1
5	76.92	1.98	80.67	78.9	53.0
6	71.43	1.54	74.83	73.65	63.0
7	76.92	1.32	80.70	79.1	58.0
8	71.43	1.54	74.83	73.65	63.0
9	76.92	2.2	80.66	78.7	48.0
10	"	4.18	80.56	78.7	49.0
11	"	1.32	80.70	76.92	46.0
12	"	8.36	80.35	78.7	52.0
13	"	55.22	78.0	77.55	58.0
14	"	47.3	78.42	77.75	56.0
15	"	53.68	78.08	77.65	63.0
16	"	12.10	80.16	78.52	49.4
17	"	67.54	77.39	77.27	74.5
18	"	68.4	77.33	77.32	100.0
19	"	17.38	79.9	78.47	52.0
20	"	78.7	79.83	78.37	50.0
21	"	23.32	79.60	78.32	52.0
22	"	34.76	79.03	78.02	52.1
23	"	40.70	78.73	77.83	50.3
24	80.0	1.98	83.9	81.92	50.0
25	70.0	20.02	72.5	71.58	63.0

Yellow

While a large degree of regularity exists in these results, it is hardly sufficient to exclude the possibility that secondary condensation products are a factor of some importance in the observed irregularity. Sirups 1-4 were strongly yellow from decomposition due to prolonged heating, and they actually show the least increase in solids by inversion.

The associate referee realized, therefore, that these irregularities could be explained conclusively only by working with pure fructose as soon as sufficient material is available, and with glucose. However, a further attempt at an explanation was made by preparing invert sugar solutions of normal polarimetric value by inversion at lower concentration and with an excess of acid to prevent the formation of reversion products—that is, a method close to analytical procedure, as follows:

Five hundred twenty grams of standard granulated sugar was dissolved in a 2 liter flask in about 1000 cc. of water, mixed with 84 grams of concentrated sulfuric acid in 500 cc. of water, and inverted by heating to 68°C. in a water bath, holding this temperature for 5 minutes, and then quickly cooling to room temperature. The solution was made up to 2 liters, exactly neutralized with barium carbonate, filtered, and analyzed as follows:

Direct polarization at 18°C. 25 cc./50 cc.  $\times 2$  =  $-34.3^{\circ}\text{V}$ .  
 Direct polarization with 5 cc. glacial acetic acid =  $-33.9^{\circ}\text{V}$ .  
 Direct polarization with 5 cc. concentrated hydrochloric acid =  $-35.05^{\circ}\text{V}$ .  
 Invert polarization with 5 cc. concentrated hydrochloric acid =  $-34.5^{\circ}\text{V}$ .  
 Ash (sulfonated) = 0.0315 per cent = 0.126 per cent of solids.  
 Brix by hydrometer =  $24.97^{\circ}$ .  
 Brix by refractometer =  $24.54^{\circ}$ .  
 Difference =  $0.43^{\circ}$ .  
 This invert solution concentrated in vacuo yielded a sirup polarizing  
 $-25.65^{\circ}\text{V}$ . at  $21^{\circ}\text{C}$ .  
 Specific gravity at  $\frac{20^{\circ}}{20^{\circ}} = 1.4404 = 83.9^{\circ}$  Brix (by pycnometer).  
 Refractometer at  $20^{\circ}$  = 85.5  
 and at another concentration:  
 Specific gravity at  $\frac{20^{\circ}}{20^{\circ}} = 1.4087 = 79.18^{\circ}$  Brix.  
 Refractometer =  $78.65^{\circ}$ .

The specific gravities of concentrated sirups are in closer agreement with the refractometer than the values at 1 : 1 dilution. This closer agreement was also observed with sirups 1-4 of Table 4.

	BRIX	REFRACTOMETER
1. Specific gravity 1.4033	= 78.35	78.45
2. Specific gravity 1.3682	= 72.9	72.9
3. Specific gravity 1.3400	= 68.5	68.3
4. Specific gravity 1.3150	= 64.4	64.0

The only conclusion from these results, contradictory in certain details, is that the total solids of invert sugar solutions as measured by refractometer are considerably lower than the figures calculable by normal hydrolysis. The results hardly permit a decision as to whether the noted irregularities are due to a lower refractive index, which is probable; to formation of reversion products under the conditions of inversion, prolonged heating and concentration; or to the combined influence of all factors. Only further work with pure crystallized fructose and glucose and densimetric and refractometric data for these sugars will solve the problem.

#### MOISTURE DETERMINATIONS.

After previous estimates as to the accuracy of drying methods with invert sugar little hope was held out for a solution in this way; however moisture was determined by various methods on sirup that was completely inverted by factory procedure with the following results:

Brix diluted 1 : 1 by hydrometer	= 79.3°
Brix diluted 1 : 1 by refractometer	= 78.5
Brix diluted 1 : 1 specific gravity 1.17643	= $39.6 \times 2 \times 79.2^\circ$
Undiluted specific gravity 1.4032	78.35
Undiluted by refractometer	78.2

*Moisture by vacuum drying at 70°C. with sand (between 40/60 mesh).*

	<i>per cent</i>	<i>per cent</i>
1 gram with sand (25 gms.) . . . . .	79.6	79.8
4 grams with sand (50 gms.) . . . . .	78.08	78.16
3 grams with sand (50 gms.) . . . . .	77.98	78.23
4 grams with sand (50 gms.) . . . . .	79.71	79.72
4 grams with sand (50 gms.) . . . . .	79.78	79.78
1 gram with asbestos . . . . .	79.6	

Sufficient constancy of weight was not obtainable even after a week's drying, when results would finally drop below even the refractometer value; 30–36 hours' drying, therefore, was considered as sufficient. Again specific gravity of the concentrated sirup is closer to the refractometer, while the specific gravity of the 1 : 1 dilution bears out the accuracy of the hydrometer values.

A modification of the Dean and Stark toluene distillation method by Bidwell and Sterling<sup>1</sup> seems to offer a new possibility for more successful moisture determination in invert sugars. The authors reported favorable results with honey and fructose.

In a trial of this method a colorless invert sirup of 75 per cent refractometer solids was used, which according to the inversion results of this report might have a true solid content of 76.7 per cent, but certainly not lower than 75 per cent.

The sirup was weighed directly into the distilling flask containing sand. The distillation with 75 cc. of toluene over a free flame was regulated according to the authors' description at 2 and later 4 drops per minute. Similar results were experienced as with honey by Bidwell and Sterling, in that there occurred considerable darkening of the sirup adhering to the walls of the flask. Contrary to the experience of these authors, however, no distinct end point could be observed, and the distillation was interrupted after about one hour, when more than 27 per cent water had gone over.

SIRUP USED	WATER DISTILLED	MOISTURE	RESIDUE
15.875 grams	4.3 cc.	27.71 per cent	dark brown

In order to avoid local superheating of the sirup on the bottom of the flask by the flame, an oil bath was used in further tests. Distillation

<sup>1</sup> *This Journal*, 1925, 8: 295.

would proceed at the required rate if the bath was kept at 155°C. After 1 hour 20 minutes only 21.9 per cent water had distilled over, but eventually the quantity again exceeded 25 per cent, and charring on the flask bottom was delayed but not prevented.

Another sucrose solution of 66.7 per cent gave the following results:

SIRUP USED	WATER DISTILLED	MOISTURE	TIME OF DISTILLATION
grams	cc.	per cent	hours
10.002	3.35	33.5	2
	3.47	34.7	4
10.02	3.45	34.4	2

Charring on the walls of the flask also occurred to a less degree with sucrose and produced too high results. In order entirely to eliminate local superheating the sirup was now weighed into a freshly ignited alundum extraction shell packed with glass wool. The shell was suspended in the flask without touching the bottom and kept entirely submerged under toluene during distillation. Although the sirup spread over the glass wool offered a larger surface to contact with boiling toluene than when mixed with sand, distillation proved to be much slower than with sand. Local superheating, therefore, had been largely responsible for the quicker result with sand. Four hours at least was required to distil over all the water in the sample, but again no end point was noticeable, and after continued distillation for 6-8 hours 26 per cent of water was obtained from the invert sirup of, at most, 25 per cent moisture. The color of the residue was light brown.

In order to accelerate the distillation xylene was tried; 23.5 per cent of water came over in 45 minutes, but again, as expected, no end point could be observed. After 5 hours, 34.2 per cent of water was obtained, though decomposition was quite evident from the dark brown color of the residue.

The toluene method, therefore, was found to have no advantage over drying. Under proper precautions it requires as much time, and the end point is by no means sharper. Vacuum drying at 100°-110°C. is quicker perhaps and probably as accurate.

It is recommended that future work consist, primarily, in the establishment of accurate densimetric and refractometric data on fructose and glucose, and that on the basis of these a critical study be made of drying methods, starting with pure sugar solutions and progressing gradually toward the more complicated mixtures that resemble the various sugar products<sup>1</sup>.

<sup>1</sup>For report of Sub-Committee A and action of the Association, see *This Journal* 1926, 9 : 73.

## REPORT ON POLARISCOPIC METHODS.

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*.

In the report<sup>1</sup> presented to this association a year ago, it was shown that in the analysis of sugar mixtures the Clerget divisor must be based not on the partial sucrose concentration, but on the total sugar concentration. It was also found that when the inversion is carried out at room temperature the methods of neutral polarization recommended by Jackson and Gillis<sup>2</sup>, and designated by them as No. II and No. IV, give correct results for sucrose and for mixtures of this sugar with pure invert sugar. But if reversion products are also present, then any method in which strong acid is used for inversion will at least partly hydrolyze the condensation products of reducing sugars. If the plain acid method is used, results too high are obtained even in the absence of reversion products, as is already well known.

It was pointed out in last year's report that the use of high temperatures for inversion may—especially in the presence of acid—lead to further complications; if the product to be analyzed contains invert sugar, inversion and reversion may go on simultaneously, and the equilibrium condition will depend on a number of factors, such as concentration of the various carbohydrates, temperature, time, and strength of acid.

In planning the work for the present year, it was decided, therefore, to ascertain what results are obtained when subjecting mixtures of sucrose and invert sugar, with or without reversion products, to the various methods of Clerget procedure at high temperatures, and to compare these results with those of the standard invertase method carried out at room temperature. In addition to this program, it was planned to make a study of the enzyme method devised in the Bureau of Chemistry for the analysis of mixtures of sucrose and raffinose.

#### A. DETERMINATION OF SUCROSE IN THE ABSENCE OF RAFFINOSE

Like last year, three materials were used in this work, as follows:

- (1) Sucrose (Domino tablet sugar);
- (2) Invert sugar in the form of a sirup prepared from sucrose by means of invertase without the use of high temperatures. This sirup was kindly made by R. T. Balch, of the Bureau of Chemistry. It will hereafter be designated as Sirup A.
- (3) A commercial invert sugar, prepared from cane sugar by inversion with acid at high temperature, and therefore containing reversion products. The associate referee is indebted to M. A. Schneller of the Nulomoline Co. for furnishing a supply of this material. As it was received in the form of a paste, it was dissolved in water to a sirup

<sup>1</sup> *This Journal*, 1925, 8: 384.

<sup>2</sup> U. S. Bur. Standards Scientific Paper 375.

of about 54 Brix, in order to facilitate the weighing out of aliquots, and the reaction was then adjusted to pH 7 by the careful addition of carbonate of soda solution. This sirup will hereafter be designated as Sirup B.

Sucrose was determined in these three products, as well as in mixtures of 1 and 2, and of 1 and 3, containing equal parts of sucrose and non-sucrose solids. It was not considered necessary this year to use other proportions than equal parts, because the effect of this factor on the Clerget divisor was definitely settled by last year's work.

#### METHODS USED.

The modifications of the Clerget method selected for study were:

(1) *Standard method*.—Invertase method at room temperature<sup>1</sup>. Basic divisor for 13 grams in 100 cc. at 20°: 132.0.

(2) *Rapid invertase method*<sup>2</sup>.—Basic divisor: 132.0.

(3) *Acid method at 67°–69.5°*<sup>3</sup>.—Basic divisor: 133.0.

(4) *Jackson and Gillis method No. I*<sup>4</sup>.—Using inversion procedure (a), at 60°. Basic divisor: 133.25.

(5) *Jackson and Gillis method No. II*.—Using inversion procedure (a), at 60°. Basic divisor: 133.34.

(6) *Jackson and Gillis method No. IV*.—Using inversion procedure (a), at 60°. Basic divisor: 132.63.

These six methods were used at one dilution only, 13 grams of solids in 100 cc., for the direct as well as for the invert reading. Other concentrations were omitted because the question of the change in the Clerget divisor with change in concentration involves the larger problem as to whether or not the commonly accepted concentration factor of 0.0676 holds true for all the different inversion procedures. This subject is in itself so large that it will be best to postpone its detailed study until the more pressing problems have been solved.

Like last year no elaborate equipment, such as would be needed for the highest attainable precision, was used in the analytical work, except that the readings were taken at 20°C. in jacketed tubes in a constant temperature room. Balch made duplicate, and sometimes triplicate determinations, while one determination each was made by C. A. Gamble and G. H. Hardin, of the New York Sugar Trade Laboratory, with occasional repetitions when satisfactory agreement was not reached in the

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 183.

<sup>2</sup> *Ibid.*, 186, 22(C).

<sup>3</sup> *Ibid.*, 23.

<sup>4</sup> U. S. Bur. Standards Scientific Paper 375.

duplicates. The figures given in the tables are the averages for each laboratory. The writer wishes to express his sincere thanks to the chemists named for their cooperation.

In three of the tables (3, 4, and 5) the percentage of sucrose found in the analyses is expressed on the basis of partial sucrose concentration, as well as total sugar concentration, and compared with the results calculated from the sucrose contained in the components of the mixture, separately for each method of inversion, as was done last year. In the case of sucrose this was, of course, not necessary. Sirup A contained so little sucrose that the small differences in the divisors do not affect the percentage of sucrose found, and even in Sirup B, where the calculations were made both ways, the differences are very slight, not exceeding 0.02 per cent.

The results of the entire investigation are assembled in Tables 1 to 5.

TABLE 1.

*Sucrose.*

ANALYST AND METHOD USED	DIRECT POLARIZATION	INVERT POLARIZATION	PERCENTAGE
<i>Invertase method, room temperature</i>			
R. T. Balch	49.93	-16.06	49.99
C. A. Gamble, G. H. Hardin	49.86	-15.96	49.86
			49.93
<i>Rapid invertase method</i>			
R. T. Balch	49.93	-16.03	49.97
C. A. Gamble, G. H. Hardin	49.86	-15.99	49.89
			49.93
<i>A. O. A. C. acid method, at 67°-69°C.</i>			
R. T. Balch	49.93	-16.52	49.96
C. A. Gamble, G. H. Hardin	49.86	-16.43	49.84
			49.90
<i>Jackson and Gillis method No. I at 60°</i>			
R. T. Balch	49.93	-16.58	49.91
C. A. Gamble, G. H. Hardin	49.86	-16.47	49.78
			49.85
<i>Jackson and Gillis method No. II at 60°</i>			
R. T. Balch	49.65	-16.86	49.88
C. A. Gamble, G. H. Hardin	49.58	-16.81	49.79
			49.84
<i>Jackson and Gillis method No. IV at 60°</i>			
R. T. Balch	49.66	-16.58	49.94
C. A. Gamble, G. H. Hardin	49.60	-16.47	49.82
			49.88

TABLE 2.

*Pure invert sugar sirup.*

ANALYST AND METHOD USED	DIRECT POLARIZATION	INVERT POLARIZATION	PERCENTAGE
<i>Invertase method, room temperature</i>			
R. T. Balch	-15.48	-15.53	0.08
C. A. Gamble, G. H. Hardin	-15.31	-15.36	0.08
			0.08
<i>Rapid invertase method</i>			
R. T. Balch	-15.46	-15.52	0.09
C. A. Gamble, G. H. Hardin	-15.31	-15.36	0.08
			0.08
<i>A. O. A. C. acid method at 67°-69°C.</i>			
R. T. Balch	-15.46	-16.01	0.83
C. A. Gamble, G. H. Hardin	-15.31	-15.73	0.63
			0.73
<i>Jackson and Gillis method No. I</i>			
R. T. Balch	-15.46	-16.07	0.92
C. A. Gamble, G. H. Hardin	-15.31	-15.91	0.90
			0.91
<i>Jackson and Gillis method No. II</i>			
R. T. Balch	-16.35	-16.38	0.04
C. A. Gamble, G. H. Hardin	-16.15	-16.13	0.00 (Neg.)
			0.02
<i>Jackson and Gillis method No. IV</i>			
R. T. Balch	-16.06	-16.07	0.02
C. A. Gamble, G. H. Hardin	-15.85	-15.91	0.09
			0.05

In the following discussion of the results, wherever comparisons are made between found and calculated values, the values found on the basis of *total sugar* concentration are always referred to, as this has been definitely proved to be the correct procedure. The apparent exceptions to this rule, found this year, will be discussed separately later.

## DISCUSSION.

The sucrose used in one of the laboratories was of a slightly lower polarization than that employed in the other. There were, likewise, slight differences in the quantities of Sirups A and B weighed out, equivalent to 13 grams of solids, owing to permissible errors in the refractometer readings. In order to take these differences into consideration, the percentage of sucrose found in each analysis of mixtures is always compared, in the tables, with that calculated from the figures obtained in the *same*



TABLE 3.  
Mixture of sucrose and pure invert sugar sirup.

ANALYST AND METHOD USED	DIRECT POLARIZA- TION	INVERT POLARIZA- TION	SUCROSE FOUND m = C	SUCROSE FOUND m = S	SUCROSE CALCULATED ON BASIS OF SAME METHOD
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<i>Invertase method, room temperature</i>					
R. T. Balch	17.23	-15.80	50.05	50.21	49.97
C. A. Gamble, G. H. Hardin	17.17	-15.70	49.80	49.97	49.89
			49.93	50.09	49.93
<i>Invertase, rapid method</i>					
R. T. Balch	17.23	-15.77	50.00	50.17	49.98
C. A. Gamble, G. H. Hardin	17.18	-15.72	49.85	50.02	49.89
			49.93	50.10	49.94
<i>A. O. A. C. acid method at 67°-69°C.</i>					
R. T. Balch	17.23	-16.28	50.39	50.56	50.35
C. A. Gamble, G. H. Hardin	17.18	-16.02	49.93	50.09	50.18
			50.16	50.33	50.27
<i>Jackson and Gillis method No. I at 60°</i>					
R. T. Balch	17.23	-16.29	50.31	50.48	50.39
C. A. Gamble, G. H. Hardin	17.18	-16.20	50.10	50.27	50.31
			50.21	50.38	50.35
<i>Jackson and Gillis method No. II at 60°</i>					
R. T. Balch	16.65	-16.61	49.89	50.05	49.95
C. A. Gamble, G. H. Hardin	16.65	-16.45	49.65	49.81	49.86
			49.77	49.93	49.91
<i>Jackson and Gillis method No. IV at 60°</i>					
R. T. Balch	16.80	-16.29	49.90	50.06	49.94
C. A. Gamble, G. H. Hardin	16.85	-16.20	49.84	50.00	49.90
			49.87	50.03	49.92

laboratory for the components. But since in one and the same laboratory the same weights or aliquots were always used, it is also perfectly permissible to base conclusions on the averages for the two laboratories.

For all the five products analyzed, the rapid invertase method gives average results in very close agreement with the standard method, the greatest difference amounting to 0.04 per cent. It is noted, however, that for the sucrose alone the average Clerget value in both of these methods is somewhat higher than the average direct polarization. If the Clerget factor 132.1 be used instead of 132.0, the agreement is closer between direct polarization and Clerget value. While in Sirups A and B the difference in these two divisor values does not affect the results, the agreement between found and calculated figures for the two mixtures would also average better with the higher divisor than with the lower.

TABLE 4.  
Commercial invert sugar sirup.

ANALYST AND METHOD USED	DIRECT POLARIZA- TION	INVERT POLARIZA- TION	SUCROSE	
			m = C	m = S
			per cent	per cent
<i>Invertase method, room temperature</i>				
R. T. Balch	-12.86	-13.42	0.85	0.85
C. A. Gamble, G. H. Hardin	-12.79	-13.36	0.86	0.87
			0.86	0.86
<i>Invertase, rapid method</i>				
R. T. Balch	-12.86	-13.41	0.83	0.84
C. A. Gamble, G. H. Hardin	-12.75	-13.37	0.94	0.95
			0.88	0.89
<i>A. O. A. C. acid method at 67°-69°C.</i>				
R. T. Balch	-12.86	-14.97	3.17	3.19
C. A. Gamble, G. H. Hardin	-12.75	-14.83	3.13	3.15
			3.15	3.17
<i>Jackson and Gillis method No. I at 60°</i>				
R. T. Balch	-12.86	-15.02	3.24	3.26
C. A. Gamble, G. H. Hardin	-12.75	-14.92	3.26	3.28
			3.25	3.27
<i>Jackson and Gillis method No. II at 60°</i>				
R. T. Balch	-13.68	-15.29	2.41	2.43
C. A. Gamble, G. H. Hardin	-13.65	-15.15	2.25	2.27
			2.33	2.35
<i>Jackson and Gillis method No. IV at 60°</i>				
R. T. Balch	-13.39	-15.02	2.46	2.48
C. A. Gamble, G. H. Hardin	-13.30	-14.92	2.44	2.46
			2.45	2.47

There is thus presented a further confirmation of previous findings in both laboratories, that the value 132.0 as the basic Clerget divisor for the invertase method adopted by this association is somewhat low and should be increased to 132.1.

#### METHODS SPECIFYING ACID.

Turning now to the four methods in which acid is used for inversion, it may be stated that it is the experience of the associate referee that these methods do not give as close agreement between duplicate analyses, even when made by the same analyst, as the invertase method. This condition is also reflected in the averages, as is clearly shown in Table 5, where the greatest difference between the findings of the two laboratories is 0.06 per cent for the invertase methods, and 0.36 per cent for the acid methods. It has likewise been noted that one analyst working with the same equipment and in supposedly the same manner will

TABLE 5.  
*Mixture of sucrose and commercial invert sugar sirup.*

ANALYST AND METHOD USED	DIRECT POLARIZA- TION	INVERT POLARIZA- TION	SUCROSE FOUND <i>m</i> = <i>C</i>	SUCROSE FOUND <i>m</i> = <i>S</i>	SUCROSE CALCULATED ON BASIS OF SAME METHOD
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<i>Invertase method, room temperature</i>					
R. T. Balch	18.54	-14.74	50.42	50.59	50.36
C. A. Gamble, G. H. Hardin	18.55	-14.70	50.38	50.55	50.29
			50.40	50.57	50.33
<i>Invertase, rapid method</i>					
R. T. Balch	18.55	-14.71	50.39	50.56	50.35
C. A. Gamble, G. H. Hardin	18.55	-14.67	50.33	50.50	50.33
			50.36	50.53	50.34
<i>A. O. A. C. acid method at 67°-69°C.</i>					
R. T. Balch	18.55	-15.76	51.59	51.76	51.52
C. A. Gamble, G. H. Hardin	18.55	-15.53	51.25	51.42	51.43
			51.42	51.59	51.48
<i>Jackson and Gillis method No. I at 60°</i>					
R. T. Balch	18.55	-15.81	51.57	51.74	51.55
C. A. Gamble, G. H. Hardin	18.55	-15.60	51.26	51.43	51.49
			51.42	51.59	51.52
<i>Jackson and Gillis method No. II at 60°</i>					
R. T. Balch	17.97	-16.13	51.15	51.32	51.14
C. A. Gamble, G. H. Hardin	18.02	-15.84	50.79	50.96	50.99
			50.97	51.14	51.07
<i>Jackson and Gillis method No. IV at 60°</i>					
R. T. Balch	18.13	-15.81	51.18	51.35	51.16
C. A. Gamble, G. H. Hardin	18.15	-15.60	50.89	51.06	51.08
			51.04	51.21	51.12

generally obtain lower results than another analyst when using acid, but will closely agree with the other when using invertase.

Although it is well known that the plain acid method at high temperature, either according to Schrefeld<sup>1</sup> or according to Jackson and Gillis, yields results too high for mixtures containing invert sugar, these methods were nevertheless included in this work plan, for the special purpose of ascertaining just what effect the acid exerts at high temperature on the invert sugar present at the beginning of the hydrolysis. It was not surprising to find, especially in the acid method at 67°-69.5°, that the condition mentioned in the preceding paragraph is particularly noticeable, simply because the inversion temperature is quite high, and slight variations in it may have a pronounced influence. The average figures for

<sup>1</sup> Z. Ver. deut. Zucker-Ind., 1920, 70: 402.

sucrose in the two mixtures (Tables 3 and 5), obtained in one of the laboratories, are considerably lower than those calculated for the same method, owing to a greater destruction of invert sugar than took place in the analysis of the invert sugar sirup alone.

Even lowering the inversion temperature to  $60^{\circ}$ , as recommended by Jackson and Gillis, does not remedy this condition, because even here the high temperature can at once affect the invert sugar already present.

It is found, therefore, that the two plain acid methods give too high results for the actual sucrose content, but they are liable to be lower than when the inversion is carried out at room temperature.

The Jackson and Gillis method No. II presents a most interesting case. It will be remembered that this method, with inversion at room temperature, gives correct results for sucrose in mixtures with pure invert sugar, but not when reversion products are also present. There was also found last year a slight tendency towards destruction of invert sugar by the ammonia used for neutralizing the acid. This tendency is again shown in this year's results, with inversion at high temperature. Even on pure sucrose the Clerget result was found a little low. With the pure invert sugar sirup one of the analysts observed a lower negative reading than before inversion, thus obtaining a negative result from destruction of invert sugar. The average for the two laboratories, therefore, is too low. In the mixture of sucrose and pure invert sugar sirup low figures were again found, and for the same reason.

However, in invert sugar Sirup B, containing reversion products, the result for sucrose is found very much higher than the actual percentage, owing to hydrolysis of the reversion products, and the same thing is, of course, also observed in the mixture of sucrose with Sirup B.

The interesting result is thus observed that the Jackson and Gillis method No. II, with inversion at  $60^{\circ}$ , tends to give low results when the invert sugar is pure, but high results when it is contaminated with reversion products. It is easy to conclude from this that under certain conditions correct results may be obtained by this method through a compensation of errors—namely, when a state of equilibrium obtains between reversion of invert sugar and hydrolysis of sucrose or reversion products.

A similar situation exists in Jackson and Gillis method No. IV. However, here only the high temperature exerts its effect, no ammonia being used. Therefore, while the average results obtained are still low in the presence of pure invert sugar, the discrepancies are not so great as in method No. II; on the other hand, in the presence of reversion products, in addition to invert sugar, the plus error over the actual percentage of sucrose is greater than in method No. II.

In the light of the results and deductions that have been presented, the question of the Clerget divisor as affected by concentration of either

sucrose or total sugars can now be considered. Tables 3 and 5 contain several apparent exceptions to the rule that the divisor must be based on the total sugar concentration. It is now clearly seen that these apparent exceptions are really caused by analytical errors due to destruction of invert sugar. This proves again how necessary it was first to investigate inversion methods at room temperature, as was done last year, because otherwise the apparent exceptions to Vosburgh's rule<sup>1</sup>, when high temperatures are used for inversion by acid, might have led to erroneous conclusions.

Summarizing the results of the work done during the past two years on this question, it has been found that:

(1) When analyzing sugar mixtures, the Clerget divisor must be based on total sugar concentration, and not on sucrose alone.

(2) The rapid invertase method, applied to sucrose and mixtures of it with invert sugar, either with or without reversion products, gives the same results as the standard invertase method at room temperature. The Clerget factor for both of these methods should be raised from 132.0 to 132.1.

(3) As is already well known, the plain acid method at 67°–69.5°, at 60°C., or at room temperature, gives too high results when invert sugar (or levulose alone) is present in any quantity.

(4) The Jackson and Gillis methods of neutral polarization, Nos. II and IV, carried out at room temperature, give correct results for sucrose alone and for mixtures with pure invert sugar; in the presence of reversion products the results are too high. The same conclusions hold for inversion at 60°, except that there is a tendency towards low results in the presence of pure invert sugar.

#### B. DETERMINATION OF SUCROSE AND RAFFINOSE BY THE TWO-ENZYME METHOD OF PAINE AND BALCH.

A practical method for the determination of both sucrose and raffinose in the presence of each other, as may occur in beet products, has been worked out by Paine and Balch<sup>2</sup>. Instead of employing hydrochloric acid for hydrolysis, as has been the practice heretofore, they use two enzyme preparations, a top yeast extract which contains invertase but must be free from melibiase, and a bottom yeast extract which contains both invertase and melibiase. The former hydrolyzes sucrose to dextrose plus levulose, and raffinose to melibiose plus levulose; the latter converts sucrose also into dextrose plus levulose, while the raffinose is split into galactose, dextrose, and levulose. Both sucrose and raffinose can be calculated from the direct polarization and the two invert read-

<sup>1</sup> *J. Am. Chem. Soc.*, 1921, 43: 219.

<sup>2</sup> *Ind. Eng. Chem.*, 1925, 17: 240; Invertase hydrolysis constants for sucrose and raffinose. Paper presented at the Baltimore Meeting of the A. C. S., April 1925.

ings obtained after hydrolysis with the two enzyme extracts. The exact method of procedure is described in detail in the papers referred to, and need not be repeated here. The authors of these papers have shown the superiority of the enzyme method over the usual acid methods in the analysis of complex mixtures such as occur in beet products. The complications arising from the effect of hydrochloric acid, and due either to hydrolysis of non-sugars or to the effect of the acid on the rotation of non-sugars, are entirely avoided; it has also been proved by this method that raffinose exists in certain beet products in which it cannot be detected by the acid method.

The importance of these findings is so apparent that it appeared desirable to initiate a collaborative study of the method under the auspices of this association, in order to decide whether or not it will be advisable to recommend its adoption by the association.

Samples of the two sugars, as well as the necessary enzymic extracts, were prepared and furnished by the Carbohydrate Laboratory of the Bureau of Chemistry. The analytical work was carried out by M. A. McCalip of the same laboratory, and by C. A. Gamble and G. H. Hardin, of the New York Sugar Trade Laboratory. The results given in Table 6 represent averages for both of the laboratories.

The sucrose used was free from ash and invert sugar; the raffinose hydrate showed an  $[\alpha]_D^{20}$  of 104.7°. The quantities of the latter sugar actually weighed for each analysis were calculated so as to contain the grams of anhydrous raffinose indicated in the table.

In carrying out the analyses, twice the weights of each sugar given in the table were dissolved to a total volume of 200 cc. The reading of the resultant solution in a 2 dm. tube at 20°C. is the direct polarization. For the two invert polarizations, two 50 cc. portions of the original solution were treated with 5 cc. of top and bottom yeast extract, respectively, and after standing overnight at room temperature finally made up to 100 cc. The readings were taken in a 4 dm. tube at 20°C., and these readings represent directly the invert polarizations.

The results of the analyses were calculated by the following formulas, proposed by Paine and Balch:

$$R = 1.354 (A - B), \text{ and}$$

$$S = \frac{(P - 2.202 A + 1.202 B) 100}{132.12 + 0.0728 (m - 13)}, \text{ where}$$

$S$  = percentage of sucrose;

$R$  = percentage of raffinose;

$P$  = direct polarization;

$A$  = invert polarization after treatment with top yeast extract;

$B$  = invert polarization after treatment with bottom yeast extract;

$m$  = grams of sucrose per 100 cc. in solutions used for inversion.

The results of the analyses are shown in Table 6.

TABLE 6.  
*Mixtures of sucrose and raffinose.*

ANALYST	SUCROSE, GRAMS/100 CC.	RAFFINOSE, GRAMS/100 CC.	DIRECT POLARIZATION, 20°C.	INVERT POLARIZATION.		SUCROSE TAKEN	SUCROSE FOUND	RAFFINOSE TAKEN	RAFFINOSE FOUND
				By top yeast, cord. 20°.	By bottom yeast, cord. 20°.				
1 M. A. McCalip C. A. Gamble G. H. Hardin	6.50	0.650	°V. 29.61	— 5.43	— 7.23	25.00	25.02	2.50	2.44
			29.59	— 5.42	— 7.23		24.98		2.45
			29.60	— 5.425	— 7.23		25.00		2.45
2 M. A. McCalip C. A. Gamble G. H. Hardin	6.50	0.260	26.83	— 6.83	— 7.56	25.00	24.94	1.00	0.99
			26.83	— 6.96	— 7.58		25.14		0.84
			23.83	— 6.895	— 7.57		25.04		0.92
3 M. A. McCalip C. A. Gamble G. H. Hardin	13.00	0.520	53.69	— 13.84	— 15.32	50.00	49.94	2.00	2.00
			53.71	— 13.88	— 15.28		50.03		1.90
			53.70	— 13.86	— 15.30		49.98		1.95
4 M. A. McCalip C. A. Gamble G. H. Hardin	19.50	0.780	80.45	— 20.96	— 23.16	75.00	74.89	3.00	2.98
			80.46	— 20.93	— 23.00		74.96		2.80
			80.455	— 20.945	— 23.08		74.93		2.89
5 M. A. McCalip C. A. Gamble G. H. Hardin	6.50	0.130	25.93	— 7.40	— 7.78	25.00	25.01	0.50	0.51
			25.95	— 7.32	— 7.65		25.00		0.45
			25.94	— 7.36	— 7.715		25.01		0.48
6 M. A. McCalip C. A. Gamble G. H. Hardin	13.00	0.260	51.85	— 14.84	— 15.57	50.00	49.99	1.00	0.99
			51.85	— 14.76	— 15.43		49.96		0.91
			51.85	— 14.80	— 15.50		49.98		0.95
7 M. A. McCalip C. A. Gamble G. H. Hardin	19.50	0.390	77.77	— 22.36	— 23.46	75.00	74.92	1.50	1.49
			77.75	— 22.38	— 23.45		74.91		1.45
			77.76	— 22.37	— 23.455		74.92		1.47

The direct polarizations obtained in the two laboratories show excellent agreement, the differences not exceeding 0.02°V. The readings after inversion with top yeast extract also check well, except in mixture No. 2, where the higher reading recorded in one of the two laboratories

caused a low figure in the raffinose percentage. For the bottom yeast extract the readings agree closely in four cases, but in the other three the results found in one of the laboratories were somewhat low. This may be due to the fact that the bottom yeast extract used there was over six months old and may have lost some of its activity. The latter extract was not tested immediately before use, no melibiose being available for the purpose.

Nevertheless, the agreement between percentages of sucrose used and sucrose found is very good, especially in the averages of the two laboratories. The raffinose percentages found in one of the laboratories check well with the theoretical in every case, and for the other laboratory in five out of the total of seven mixtures; the averages are satisfactory in every case.

#### RECOMMENDATIONS<sup>1</sup>.

On the basis of the results obtained during the past two years, the following recommendations are offered:

(1) That the methods of polariscopic analysis in the absence of raffinose adopted last year be finally adopted by this association.

(2) That the basic divisor for both the standard and the rapid invertas. method, used in the absence of raffinose, be increased from 142.0 to 142.1.

(3) That the method of determining sucrose and raffinose in the presence of each other, by the two-enzyme procedure of Paine and Balch, be adopted.

It is further recommended—

(4) That the investigation presented under A (Determination of sucrose in the absence of raffinose) be continued, sucrose, invert sugar, reversion products, and also other non-sugars occurring in saccharine products not derived from the beet, being used.

(5) That the investigation presented under B (Determination of sucrose and raffinose by the two-enzyme method of Paine and Balch) be repeated next year, the quantities of raffinose used being extended beyond this year's limit of 3 per cent.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9 73



## REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS.

By R. F. JACKSON (Bureau of Standards, Washington, D. C.),  
*Associate Referee.*

A notable tendency shown in recent literature respecting reducing sugar analysis is in the development of methods for the selective determination of the individual sugars. The most recent of these methods is one proposed by Nyns<sup>1</sup>, who has made the observation that Ost's copper carbonate solution is reduced at 48.5° to 49°C. by levulose alone. The author states that the reagent is unaffected by glucose or other sugars except a few pentoses. The associate referee has made a preliminary study of this method, and while he is unable to confirm the author's claim that the reagent is quite indifferent to the presence of glucose, he believes that it has sufficient merit to warrant an extended investigation. He finds that while about 3 mg. of levulose will reduce 10 mg. of copper, it requires about 50 mg. of dextrose to reduce 10 mg. of copper. Consequently, if the analyst has even an approximate knowledge of the glucose content of his sample he can correct for its reducing power with a fairly high degree of certainty. The associate referee recommends that this method be studied for the purposes of this association.

Dextrose can be determined selectively by its reducing action on iodine in weakly alkaline solution. In the analysis of pure sugar mixtures but little difficulty is encountered. Unfortunately, however, iodine is reduced by a great variety of substances so that its application to crude substances and particularly to crude plant juices yields too high values for dextrose. This objection, which at present appears insurmountable, limits the usefulness of the method.

## RECOMMENDATIONS.

1. Perhaps the greatest single need among reducing sugar methods is that of a rapid and accurate volumetric procedure. In paragraphs 30-33 of the present book<sup>2</sup> are given two tentative volumetric processes, the first of which is designated "approximate." The second of these methods requires a continued repetition of the entire analysis until finally the filtrates from two precipitations differing slightly in amounts of solution added show, respectively, the presence and the absence of copper. It is believed that this procedure is too tedious to merit a place in the association's book of methods.

<sup>1</sup> C. A., 1925, 19: 1236.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 189.

In 1923 Lane and Eynon<sup>1</sup> developed a volumetric method based upon Soxhlet's modification of Fehling's solution and a basic period of two minutes' boiling, the final titration being subsequently completed within one minute. The end point of the reduction is determined by the decolorization of a few drops of 1 per cent methylene blue employed as an internal indicator. The change in the indicator is very sharp, can be readily observed in the presence of the precipitate, and occurs at the same point in the reaction as potassium ferrocyanide. It involves no interruption of the boiling and consequently permits the analysis to be carried out exactly as standardized. The method is at least as accurate as the gravimetric methods and incomparably more rapid. The authors have prepared tables for pure dextrose, invert sugar, levulose, lactose, and maltose, and for sucrose-invert sugar mixtures. The associate referee has studied this method in detail and is able to confirm the author's table for dextrose, levulose, and invert sugar very closely, but recommends that as usual the individual analyst establish his own factors. The standardization for this method is particularly simple, since the whole range of factors for each sugar varies but a few per cent, and a standardization at one or two concentrations of sugar permits a correction of the whole table for variations of individual procedure and of reagents.

The associate referee has not yet had opportunity to verify the tables for lactose, maltose, or for sucrose-invert sugar mixtures and therefore is unable at this time to recommend the method as official, but he does recommend that the method be adopted as tentative and that the tentative volumetric method given in paragraphs 32 and 33 be discarded.

In his previous report<sup>2</sup> the associate referee announced a program designed to eliminate the extensive duplication in the present book of methods. This program is a rather large one, and the necessary study is still incomplete. One recommendation, however, can be made at this time.

2. There are in paragraphs 40-42 three electrolytic methods for the determination of copper, one from sulfuric acid, one from nitric acid, and one from mixtures of these two acids. The preparation of the solution for electrolysis from sulfuric acid involves a tedious evaporation for the removal of the nitric acid used to dissolve the cuprous oxide. Electrolysis from pure nitric acid is hazardous because of the danger of resolution. On the other hand, electrolysis from the mixed acids gives the most adherent deposit, and is the most convenient and advantageous. The presence of sulfuric acid, moreover, would insure the precipitation of most of the lead, which is the only probable metallic impurity. It

<sup>1</sup> *J. Soc. Chem. Ind.*, 1923, 42: 32. The copy of the method arranged for this report was published in the report of the Committee on Editing Methods of Analysis, *This Journal*, 1926, 9: 35

<sup>2</sup> *This Journal*, 1925, 8: 402.

also removes much of the danger from the oxides of nitrogen. It is recommended, therefore, that in place of the three alternative electrolytic methods the method of electrolysis from mixed sulfuric and nitric acids be retained as official and the two remaining methods be discarded<sup>1</sup>.

#### COMMITTEES NAMED BY THE PRESIDENT.

##### *Board of Editors:*

R. B. Deemer, whose term expired, was reappointed.

##### *Committee on Definitions of Terms and Interpretation of Results on Fertilizers:*

C. H. Jones was appointed to replace E. G. Proulx, deceased.

##### *Committee to Wait upon Secretary of Agriculture:*

A. J. Patten and H. J. Patterson.

##### *Committee to Wait upon Honorary President:*

W. B. Ellett and J. K. Haywood.

##### *Committee on Resolutions:*

G. S. Fraps, W. W. Randall, and Julius Hortvet.

##### *Auditing Committee:*

H. B. McDonnell and J. B. Weems.

##### *Committee on Nominations:*

F. P. Veitch, A. G. McCall, and H. H. Hanson.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 73.

**FIRST DAY.**  
**MONDAY—AFTERNOON SESSION.**

**REPORT ON FERTILIZERS.**

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.),  
*Referee.*

The Referee on Fertilizers wishes first to endorse the recommendations of the Associate Referees on Phosphoric Acid, Potash, and Nitrogen.

Other matters in connection with the methods for fertilizers that should be considered and discussed are the following:

The three methods for the preparation of ammonium citrate solution give slightly different results. It would be desirable to reduce this number.

The absolute or cupric oxide method is no longer used for fertilizers. While it is a valuable method for nitrogen in organic compounds, it now appears out of place in connection with fertilizers.

The permanganate is sometimes completely decolorized in the neutral permanganate method for nitrogen. It should be decided whether or not these results should be discarded and the analysis made on a slightly smaller quantity of material.

The use of 46.7 mg. of nitrogen in place of 50 mg. in this permanganate method would simplify the calculations and save much time in the work. If fifth-normal acid is then used, 1 cc. of acid equals 6.0 per cent of permanganate-insoluble nitrogen, instead of 5.6 per cent when 50 mg. is taken. It would be desirable to have a table showing the quantity of fertilizer to use for different quantities of water-soluble nitrogen.

At the suggestion of J. B. Weems of Virginia the following method for chlorine in mixed fertilizers is included in this report:

**CHLORINE IN MIXED FERTILIZERS.**

*Reagents.*—See method for chlorine in plants<sup>1</sup>.

Weigh 3.55 grams into a beaker and add 3 grams of calcium carbonate free from chlorine. Heat to boiling and boil 10 minutes, or until the odor of ammonia has disappeared; filter, and wash into a 200 cc. flask. Make up to volume, take 50 cc., make acid with nitric acid (d), and titrate as in 15.

With 0.1 N silver nitrate, 1 cc. is equivalent to 4 per cent of chlorine (Cl) on the amount of fertilizer taken. It is possible that the solution could be titrated directly with silver nitrate and chromate. A blank should be made on the calcium carbonate.

Attention is called to the fact that a number of changes in the methods of solution were finally adopted in 1924<sup>2</sup>. As it is desirable to have these methods printed as revised, they are given as follows:

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 44, 13.

<sup>2</sup> *This Journal*, 1925, 8: 262.

## PREPARATION OF SOLUTION.

Treat 2 grams of the sample by one of the methods given below. Cool the solution and dilute to 200 cc. Mix and pour on a dry filter.

(a) Dissolve in 30 cc. of strong nitric acid and 3-5 cc. of strong hydrochloric acid and boil until the organic matter is destroyed. This method is suitable for organic material like cottonseed meal alone or in mixtures.

(b) Dissolve in 15-30 cc. of strong hydrochloric acid and 3-10 cc. of strong nitric acid. This method is recommended for fertilizers containing much iron or aluminium phosphate.

(c) Evaporate with 5 cc. of magnesium nitrate, ignite, and dissolve in strong hydrochloric acid. This method is suitable for organic material like cottonseed meal alone or in mixtures.

(d) Boil with 20-30 cc. of concentrated sulfuric acid in a 200 cc. flask, adding 2-4 grams of sodium or potassium nitrate at the beginning of the digestion and a small quantity after the solution has become nearly colorless, or adding the nitrate in small portions from time to time. When the solution is colorless add 150 cc. of water and boil for a few minutes. This method is generally applicable to materials or mixtures containing large quantities of organic matter. With cottonseed meal and materials of like nature, it is best to add first about 5 cc. of strong nitric acid, then add the sulfuric acid, and digest at a gentle heat until the violence of the reaction is over, before beginning to add the nitrate.

## RECOMMENDATION.

The referee believes that further consideration should be given to the points enumerated in this report before definite recommendations are made.

## THE GRAVIMETRIC DETERMINATION OF PHOSPHORIC ACID.

By WILLIAM H. ROSS (Bureau of Soils, Washington, D. C.), *Associate Referee*.

At last year's meeting of this association a report<sup>1</sup> was given on the influence of the reaction of the solution to which the magnesia mixture is added on the gravimetric analysis of phosphates. The results submitted showed some variation according as the solution was made alkaline, neutral, or acid preparatory to precipitation with magnesia mixture, but the differences were less than those reported by Larison<sup>2</sup>, Caro<sup>3</sup>, and McCandless and Burton<sup>4</sup>, and best results were obtained with neutral solutions rather than with acid solutions as found by Larison and by Caro. It was accordingly recommended and approved that a collaborate study be made of this subject and that special attention also be given to the advisability of replacing the official alkaline magnesia mixture with acid mixture as recommended by the Bureau of Standards.

<sup>1</sup> Ross, Jones and Merz. *This Journal*, 1925, 8: 407.

<sup>2</sup> *This Journal*, 1924, 7: 394.

<sup>3</sup> *Am. Fertilizer*, 1924, 61, No. 6: 22.

<sup>4</sup> *Ind. Eng. Chem.*, 1924, 16: 1267.

Two standard phosphate samples were selected for this work. One was the standard phosphate rock of the Bureau of Standards, which is claimed to have a true  $P_2O_5$  content of 31.33 per cent as determined by double precipitation with magnesia mixture and for which a single precipitation gave a value of 31.45 per cent. The other sample was a synthetic calcium phosphate prepared by the associate referee. In the preparation of this sample C. P. phosphoric acid was recrystallized<sup>1</sup> three times, and the crystals were dried by allowing to stand over phosphorus pentoxide for six months. The freedom of the crystals from moisture and all other impurities was shown by the constancy of their melting point, which remained fixed at 42.35°C. throughout the period of melting, and by their rate of crystallization, which was 33.3 cm. per minute at 20°C.<sup>2</sup> Both of these crystal constants undergo a marked change on the addition of a slight amount of moisture or other impurity. The purity of the crystals was also confirmed by volumetric and gravimetric analysis. A weighed portion was dissolved in water and added to an equivalent amount of a thin paste of phosphorus-free calcium oxide. A slight excess of calcium carbonate was finally added, and the resultant mixture was carefully evaporated to dryness and then dried to constant weight at 105°C. The phosphoric acid ( $P_2O_5$ ) content of the dried material, which was calculated to be 43.94 per cent, was thus determined independently of any method of analysis. All weighings were made on a high-grade analytical balance of 1 kilo capacity and sensitive to 0.1 mg. The purity of the lime used in the preparation of the sample was of no significance inasmuch as it was found that the final product could be dried to the same constant weight on different days.

The instructions sent to the different collaborators were as follows:

Determine total phosphoric acid in each of the two samples submitted by the following procedures:

(1) Dry sample at 105°C. for 1 hour. Dissolve 2 grams of the sample in 30 cc. of hydrochloric acid (sp. gr. 1.19) and 10 cc. of nitric acid (sp. gr. 1.42), and evaporate to dryness or to a sirupy consistency. Redissolve in 5 cc. of concentrated nitric acid and 50 cc. of water. Cool the solution, make up to the mark in a graduated flask, allow to settle or pour on a dry filter, and proceed as described in the official gravimetric method for the determination of total phosphoric acid until the molybdate precipitate is washed with ammonium nitrate solution. Then dissolve the precipitate on the filter with dilute ammonium hydroxide (100 cc. ammonium hydroxide (sp. gr. 0.90) per liter of water) and wash with hot water into a beaker to a bulk of not more than 100 cc. Carefully neutralize the ammoniacal solution with hydrochloric acid, using litmus paper or brom thymol blue as indicator. Add the official magnesia mixture and complete the determination as described in the official method. It is recommended that the magnesium phosphate precipitate be filtered through paper and ignited in a platinum or porcelain crucible to constant weight at 1000°C.

<sup>1</sup> Ross, Jones and Durgin. *Ind. Eng. Chem.*, 1925, 17: 1081.

<sup>2</sup> Ross and Jones. *J. Am. Chem. Soc.*, 1925, 47: 2165.

(2) Proceed as in (1) through the point where the ammoniacal solution of the molybdate precipitate is carefully neutralized. Then make alkaline by the addition of 1 cc. of ammonium hydroxide (sp. gr. 0.90), or its equivalent, proceed with the addition of the official magnesia mixture, and complete the determination as in (1).

(3) Proceed as in (1) through the point where the solution of the molybdate precipitate is carefully neutralized. Then make acid by the addition of 1 cc. of hydrochloric acid (sp. gr. 1.19), or its equivalent, proceed with the addition of the official magnesia mixture, and complete the determination as in (1).

(4) Prepare acid magnesia mixture as follows:

Dissolve 50 grams of  $MgCl_2 \cdot 6H_2O$  and 100 grams of  $NH_4Cl$  in 500 cc. of water. Add ammonium hydroxide in slight excess, let stand overnight, and filter if a precipitate appears. Make barely acid with hydrochloric acid and dilute to 1000 cc.

Prepare solution of sample and proceed as in (1) through the point where the solution of the molybdate precipitate is carefully neutralized. Add 1 cc. of hydrochloric acid (sp. gr. 1.19) and 20 cc. of acid magnesia mixture per decigram of phosphoric acid ( $P_2O_5$ ) present. Add ammonium hydroxide (sp. gr. 0.90) dropwise and with continuous stirring until the solution is ammoniacal and most of the phosphoric acid has been precipitated. Finally add 15 cc. more of ammonium hydroxide at one time, let stand for 4 hours, and complete the determination as in (1).

#### DISCUSSION.

An examination of Table 1 shows that each of the four procedures gave best results in the hands of one or more collaborators. As a rule each of the collaborators reported different results with the different procedures, but these differences are not so marked as those occurring among the values found by the different collaborators with any given procedure.

Lower results were usually obtained with an alkaline solution than with the other procedures, but this was not always true, and while high results were usually obtained with the Bureau of Standards method, three of the collaborators report good results with this method.

Four of the collaborators expressed a preference for acid magnesia mixture, but the remaining seven preferred the official alkaline mixture.

A few of the collaborators reported that some of the magnesium pyrophosphate residues failed to burn white and that when such was the case the results were usually high. After the work was completed it was learned that some of the collaborators used 85 per cent molybdic acid while the others used the 99.9 per cent reagent.

In submitting their report, McCandless and Burton expressed the opinion that true results are obtained only when the solution is made neutral before precipitating with magnesia mixture and the residues blasted to constant weight. Therefore, values of 31.26 and 43.76 per cent, which they found for the two samples by this procedure, are considered by them to be more reliable than the results they obtained by igniting at  $1000^\circ C.$ , as given in Table 1.

The results reported by the different collaborators are given in Table 1.

TABLE 1.  
*Analysis of standard phosphate samples.*

COLLABORATORS	PHOSPHORIC ACID ( $P_2O_5$ ) FOUND							
	Phosphate Rock*				Synthetic Phosphate†			
	Official method—reaction of solution before precipitating with magnesia mixture			Modified Bureau of Standards routine method	Official method—reaction of solution before precipitating with magnesia mixture			Modified Bureau of Standards routine method
	Alkaline	Neutral	Acid		Alkaline	Neutral	Acid	
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
H. Y. Fisher Pennsylvania Department of Agriculture	31.36	31.53	31.72	31.95	43.90	44.06	43.88	44.69
J. E. Breckenridge American Agricultural Chemical Co.	31.48	31.50	31.56	31.74	43.52	43.75	43.94	43.94
A. R. Merz Bureau of Soils	31.47	31.63	31.78	31.83	43.78	43.91	43.94	44.18
G. E. F. Lundell Bureau of Standards	30.93	31.45	31.57	31.45	43.04	43.54	44.00	43.86
R. B. Deemer Bureau of Plant Industry	..	....	....	....	43.73	43.94	44.22	44.20
P. McG. Shuey Shuey & Co.	30.91	30.97	31.14	31.51	43.37	43.28	43.39	44.13
McCandless and Burton McCandless Laboratory	31.55	31.79	31.70	31.82	44.23	44.17	44.54	44.36
E. R. Tobey Maine Agricultural Experiment Station	31.76	32.08	31.88	32.00	44.12	44.32	44.18	44.82
E. L. Larison Anaconda Copper Mining Co.	30.61	30.88	31.10	31.26	42.57	43.00	43.34	43.50
R. E. Ingham F. S. Royster Guano Co.	32.22	32.08	30.36	....	44.44	44.68	43.38	44.68
C. W. Jackson F. S. Royster Guano Co.	32.08	32.22	32.56	32.73	46.66	44.56	44.68	44.56
Mean for all collaborators	31.44	31.61	31.54	31.81	43.94	43.93	43.95	44.26
Mean for selected collaborators	31.28	31.46	31.58	31.72	43.65	43.81	43.99	44.19

\* Phosphoric acid ( $P_2O_5$ ) present—31.45 per cent.

† Phosphoric acid ( $P_2O_5$ ) present—43.94 per cent.



Breckenridge recommends that the official method be modified so as to provide for the elimination of silica when this is present by evaporating the acid solution of the sample to dryness or a sirupy consistency, as outlined in the accompanying directions. Failure to remove silica in the case of the phosphate rock sample gave a difference in the results of about 0.4 per cent.

In a lengthy report submitted by Lundell of the Bureau of Standards it is pointed out that alkaline solutions gave values of 30.93 and 43.04 per cent, respectively, for the rock and synthetic phosphate samples when the magnesia mixture was added at the rate of one drop per second as specified in the official methods, and of 31.58 and 43.89 per cent when the magnesia mixture was added all at once. The first results are low while the latter are in good agreement with the theoretical. It is considered, therefore, that the rate at which the magnesia mixture is added has an important bearing on the results obtained by the official method, particularly if the solution reacts alkaline before precipitating.

Owing to the lack of agreement in some of the results, a selected mean is given in Table 1 in addition to the straight mean of all the results reported for each procedure. For the selected mean there was included the results of those collaborators who reported at least one value for each sample which agreed within 0.20 per cent of the theoretical. These selected mean values show that in the hands of these collaborators lowest results are obtained with alkaline solutions and highest with acid, but the differences are not great, as was reported last year, and both neutral and acid solutions give results which agree very well on an average with the assumed true values of the samples. It may be concluded, therefore, that while the reaction of the solution before precipitating with magnesia mixture may be a contributing source of error, it is not the principal cause of the wide variation found by different analysts.

From the results submitted it is apparent that all the collaborators do not use the same quality of reagents or follow exactly the same procedure of analysis. The writer, therefore, agrees with those who maintain that more specific directions should be given in the official methods for the determination of phosphoric acid, but as there is still a difference of opinion as to what these specific directions should be, it is recommended that the work be continued next year with the use of pure reagents and with special reference to the effect on the results of varying the conditions under which precipitation is made with magnesia mixture<sup>1</sup>.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 74.

REPORT ON NITROGEN<sup>1</sup>.

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*.

The Devarda alloy method<sup>2</sup> was adopted last year as a tentative method to determine nitrogen in nitrate salts. At the suggestion of the General Referee on Fertilizers, a new line of work was carried on this year pertaining to the methods for determining nitric nitrogen. While there are several methods either official or tentative that work very well for the determination of nitrogen in nitrate salts, these methods may give varying results when applied to mixed fertilizers containing organic nitrogen. One method in particular, the zinc-iron method<sup>3</sup>, has caused much trouble and confusion among chemists for a number of years. Most chemists have found this method impossible to use on mixed fertilizers containing organic nitrogen. The difficulty arises from the fact that strong alkali is used in this method with the result that organic matter is decomposed and high results are produced.

The method is satisfactory for nitrate salts alone, but this distinction is not made in the method as published, and therefore much confusion is created among those not familiar with methods for nitric nitrogen determinations. Furthermore, when calcium cyanamide is present in a mixed fertilizer, it is quite markedly decomposed by all the methods for nitric nitrogen, with the result that high and incorrect values are obtained. This is especially true with the zinc-iron method.

It seemed very important, therefore, to obtain data upon the points mentioned above, and to attempt to formulate a better method for determining mineral and organic nitrogen in mixed fertilizers. The work this year was entirely preliminary, and carried out by the associate referee without collaborative assistance. If the recommendations from this preliminary work are adopted, collaborative work will be undertaken next year.

Although organic materials such as tankage, dried blood, fish, and calcium cyanamide have only small amounts of nitric nitrogen, if any, it was necessary to find out what effect the various methods for determining nitric nitrogen would have on these organic materials.

The methods used in the analyses were the following:

1. Total nitrogen by the regular Kjeldahl method or modified Kjeldahl method to include nitrates<sup>4</sup>.

<sup>1</sup> Paper No. 286 of the Journal Series, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

<sup>2</sup> *This Journal*, 1925, 8: 410.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 11.

<sup>4</sup> *Ibid.*, 6 and 8.

2. Nitric and ammoniacal nitrogen by the reduced iron method<sup>1</sup>.

3. Nitric and ammoniacal nitrogen by the zinc-iron method<sup>1</sup>.

4. Nitric and ammoniacal nitrogen by the Breckenridge method<sup>2</sup>. In this method 8 grams of the fertilizer is dissolved in 200 cc. of water and filtered. A 25 cc. aliquot (equal to 1 gram) is taken and the procedure as under Par. 34<sup>1</sup> is followed.

5. Nitric and ammoniacal nitrogen by Devarda alloy method<sup>3</sup>. The solution is obtained as in the Breckenridge method.

6. Nitric and ammoniacal nitrogen in the water extract (leached) by reduced iron method<sup>2</sup>. In this method and the following one, 8 grams of the fertilizer was placed on a filter paper in a funnel and leached out with small quantities of water to a volume of 200 cc. A 25 cc. aliquot (equal to 1 gram) was taken for analysis.

7. Nitric and ammoniacal nitrogen (leached) by zinc-iron method<sup>2</sup>.

TABLE 1.

*Nitric and ammoniacal nitrogen in some organic materials by various methods.*

MATERIALS USED	1 TOTAL NITROGEN (REGULAR KJELDHAL METHOD)	2 NO <sub>3</sub> + NH <sub>3</sub> BY RE- DUCED IRON METHOD	3 NO <sub>3</sub> + NH <sub>3</sub> BY ZINC- IRON METHOD	4 NO <sub>3</sub> + NH <sub>3</sub> BY BRECKENRIDGE METHOD	5 NO <sub>3</sub> + NH <sub>3</sub> BY DEVAR- DA ALLOY METHOD (SOLUTION OBTAINED BY BRECKENRIDGE METHOD)	6 NO <sub>3</sub> + NH <sub>3</sub> IN WATER EXTRACT (LEACHED) BY REDUCED IRON METHOD	7 NO <sub>3</sub> + NH <sub>3</sub> IN WATER EXTRACT (LEACHED) BY ZINC-IRON METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Tankage	8.872	0.264	0.836	0.088	0.057	0.057	0.132
	8.762	0.201	0.727	0.075	0.082	0.060	0.173
	8.853	0.198	0.909	0.072	0.075	0.057	0.145
Average	8.811	0.221	0.824	0.078	0.071	0.058	0.150
Dried Blood	13.47	0.478		0.079	0.088	0.069	0.120
	13.49	0.469	0.727	0.079	0.075	0.101	0.132
	13.47	0.465	0.618	0.079	0.085	0.075	0.113
Average	13.48	0.471	0.673	0.079	0.083	0.082	0.122
Fish	7.891	0.912	0.982	0.717	0.648	0.682	0.799
	7.854	0.987	1.127	0.692	0.676	0.679	0.786
	7.963	0.818	0.909	0.686	0.698	0.670	
Average	7.903	0.906	1.006	0.698	0.674	0.677	0.793
Calcium cyanamide	15.20	6.655	9.816	5.072	7.526	5.157	7.108
	15.15	6.489	9.560	4.380	7.546	5.264	7.308
	15.12	6.306	9.926	5.163	7.473	5.419	7.288
Average	15.16	6.483	9.767	4.872	7.515	5.328	7.235

<sup>1</sup> *Methods of Analysis*, 1925, 11.

<sup>2</sup> *Ind. Eng. Chem.*, 1925, 17: 95.

<sup>3</sup> *This Journal*, 1925, 8: 410.

TABLE 2.

*Nitric and ammoniacal nitrogen in some mixed fertilizers by various methods.*

MIXED FERTILIZERS*	1	2	3	4	5	6	7
	TOTAL NITROGEN (KJELDAHL METHOD TO INCLUDE NITRATES)	NO <sub>3</sub> + NH <sub>3</sub> BY RE- DUCED IRON METHOD	NO <sub>3</sub> + NH <sub>3</sub> BY ZINC-IRON METHOD	NO <sub>3</sub> + NH <sub>3</sub> BY BRECKENRIDGE METHOD	NO <sub>3</sub> + NH <sub>3</sub> BY DEVAR- DA ALLOY METHOD. (SOLUTION OBTAINED BY BRECKENRIDGE METHOD)	NO <sub>3</sub> + NH <sub>3</sub> BY WATER EXTRACT (LEACHED) REDUCED IRON METHOD	NO <sub>3</sub> + NH <sub>3</sub> IN WATER EXTRACT (LEACHED) ZINC-IRON METHOD
	per cent	per cent	per cent	per cent	per cent	per cent	per cent
No. 1, approximately 4-8-3, N as Ca(NO <sub>3</sub> ) <sub>2</sub> and (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> Average	4.181	3.727	3.324	3.688	3.815	3.688	3.927
	4.200	3.727	3.828	3.744	3.815	3.701	3.886
	4.163	3.744	3.264	3.716	3.800	3.701	3.857
	4.181	3.733	3.472	3.716	3.810	3.697	3.890
No. 2, approximately 4-8-3, N as Ca(NO <sub>3</sub> ) <sub>2</sub> and CaCN <sub>2</sub> Average	4.200	2.709	3.406	2.758	2.914	2.731	3.462
	4.200	2.764	3.546	2.787	2.914	2.703	3.504
	4.200	2.836	3.858	2.787	2.942	2.745	3.476
	4.200	2.770	3.603	2.777	2.923	2.726	3.481
No. 3, approximately 4-8-3, N as Ca(NO <sub>3</sub> ) <sub>2</sub> , (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and cotton- seed meal Average	3.054	2.800	2.676	2.674	2.872	2.661	2.815
	3.018	2.818	2.758	2.843	2.927	2.745	2.801
	3.054	2.818	2.786	2.857	2.899	2.731	2.843
	3.042	2.812	2.740	2.791	2.899	2.712	2.820
No. 4, approximately 4-8-3, N as Ca(NO <sub>3</sub> ) <sub>2</sub> and tankage Average	4.200	2.673	2.590	2.604	2.646	2.491	2.661
	4.163	2.690	2.676	2.604	2.646	2.491	2.618
	4.254	2.655	2.702	2.575	2.632	2.505	2.632
	4.206	2.673	2.656	2.594	2.641	2.496	2.637
No. 5, approximately 5-8-6, N as Ca(NO <sub>3</sub> ) <sub>2</sub> and fish Average	4.837	3.218	3.660	3.083	3.322	3.166	3.561
	4.781	3.272	3.464	3.040	3.309	3.166	3.533
	4.726	3.272	3.632	3.125	3.309	3.153	3.575
	4.781	3.254	3.585	3.083	3.313	3.160	3.556

\* Peat was used as a filler in these mixtures and consequently adds to the nitrogen content.

## DISCUSSION.

In Table 1 the results obtained on some of the organic materials used in mixed fertilizers are given. The data in columns 2 and 3 on the reduced iron and zinc-iron methods show that a considerable amount of the organic material is converted into ammonia. This is no doubt due to the alkali used in the determination. With the zinc-iron method the decomposition is by far the greatest. This condition would be expected from the strength of the alkali used in this method. Breckenridge's method, column 4, and the Devarda alloy method, column 5, apparently obviated this error in a large measure. This is due chiefly to the fact that soluble organic matter only is present in the solution to be analyzed for nitric nitrogen, while in the other methods the whole material is present. It is also true that when there is much soluble organic material

in the substance to be analyzed, it is partially decomposed by the alkali used and gives high results.

Calcium cyanamide is decomposed by any of these methods, as shown by the results in Table 1, and in proportion to the strength of the alkali.

The data in column 6 are in close agreement with those in column 4, as would be expected, since the two methods of procedure are nearly the same. Column 7 is interesting because the zinc-iron method produced relatively high results on account of the action of strong sodium hydroxide on the soluble organic nitrogen, even though the fertilizers were leached out.

Table 2 records the data obtained by these methods on mixed fertilizers. If organic materials are used in mixed fertilizers, there is the same tendency to decomposition, especially by the zinc-iron method; it is less noticeable, however, owing to the smaller fraction of organic material present.

Another very serious fault with the zinc-iron method lies in the fact that excessive frothing, produced when organic matter is present, makes the determination extremely difficult to carry out.

### CONCLUSIONS.

It is quite evident from the data shown in the tables that the zinc-iron method causes the greatest decomposition of the organic material, thus making its use impracticable for determining nitric nitrogen where organic matter is present.

The reduced iron method shows similar tendencies but to a much less degree.

The Breckenridge method, which is really only a simple modification of the reduced iron method, gave the most satisfactory results and the least decomposition of organic matter. This method, however, does not eliminate the effects of soluble organic nitrogen.

Calcium cyanamide is decomposed by all these methods, but least by the Breckenridge method. When it is present with nitrates in organic mixed fertilizers, a new procedure or method for determining the nitric nitrogen is necessary, but this problem still remains to be worked out.

The results obtained by the associate referee show clearly that the zinc-iron method should either be eliminated from the official methods of analysis, or be put under the heading "Nitrogen in Nitrate Salts".

RECOMMENDATIONS.<sup>1</sup>

It is recommended—

(1) That the zinc-iron method be placed under the heading “nitrogen in nitrate salts” as it is unsuitable for mixed fertilizers.

(2) That collaborative work be done on the Breckenridge method for inorganic nitrogen in mixed fertilizers.

(3) That further study be made with a view to devising an accurate method for inorganic nitrogen in mixed fertilizers when calcium cyanamide is present.

## REPORT ON POTASH.

By A. P. KERR (Agricultural Experiment Station, Baton Rouge, La.),  
*Associate Referee.*

The association recommended at its 1924 meeting that the method for the precipitation of phosphorus in the water-soluble solution of potash fertilizers be studied. The following procedure was carried out in comparison with the A. O. A. C. method<sup>2</sup>, by the different chemists collaborating.

Weigh 2.5 grams of sample, place on a 12.5 filter paper, and wash with boiling distilled water into a 250 cc. flask up to about 200 cc. Make alkaline with ammonium hydroxide (1 + 1).

Add 25 cc. of magnesium chloride solution (5 grams to 100 cc. of water) and then add 5 cc. of ammonium oxalate solution (2 grams to 100 cc. of water) or enough to precipitate all the lime present. Let cool, make up to mark, and shake well. Allow to stand until the precipitate settles. Draw off the clear solution and filter. Take a 50 cc. aliquot, add about 5 cc. of sulfuric acid (1 + 1), and evaporate to dryness. Burn off organic matter, heating to red heat. Take up with boiling water, filter into an evaporating dish, and add a few drops of hydrochloric acid.

Add platinum solution as described in the A. O. A. C. method. Also wash the precipitate  $K_2PtCl_6$  as described in the official method.

Use 95 per cent alcohol on half of determinations.

Use 80 per cent alcohol on half of determinations.

Report separate results on each procedure.

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<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1925, 9. 74.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 13.

*Collaborative results.*

COLLABORATOR	A. O. A. C. METHOD	PHOSPHORUS PRECIPITATED WITH $MgCl_2$ AND $K_2PtCl_6$ WASHED WITH 80 PER CENT ALCOHOL	PHOSPHORUS PRECIPITATED WITH $MgCl_2$ AND $K_2PtCl_6$ WASHED WITH 95 PER CENT ALCOHOL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
W. F. Hand, Agricultural and Mechanical College, Agricultural College, Miss.	5.54	5.67	5.75
J. W. Kellogg, Department of Agriculture, Harrisburg, Pa.	5.72	5.98	5.93
L. J. Savana, Armour Fertilizer Works, New Orleans, La.	5.61	5.69	....
C. M. Bible, Read Phosphate Co., Nashville, Tenn.	5.69	5.60	5.63
E. S. Asbury, Agricultural Experiment Station, College Station, Tex.	5.58	6.45	6.42
A. P. Kerr	5.58	5.66	5.71

The sample of fertilizer used in this work consisted of a mixture of acid phosphate and potassium chloride containing 5.67 per cent of potassium oxide.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the study of the method requiring magnesium chloride is not promising and should be discontinued.

(2) That further study be made on methods for the prevention of the formation of metaphosphates, as they probably cause errors in the results.

## A METHOD FOR POTASH IN MIXED FERTILIZERS.

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.).

The procedure in the method here submitted is intended to accomplish several objects. First it renders the phosphoric acid insoluble before the potash is extracted, thereby avoiding the presence of phosphoric acid in the filtrate. Second, it avoids the precipitation with ammonia and ammonium oxalate, which may cause absorption of potash and which usually leaves phosphoric acid in solution. Third, it does not require ignition, thereby making the use of quartz or platinum dishes unnecessary, saving time, and avoiding possible loss by spitting and the formation of metaphosphates. The method is based upon experimental work on the effect of carbonate of lime upon phosphoric acid in mixed fer-

<sup>1</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 74.

tilizers and on the De Roode method for potash, which is so successful when applied to soils.

#### DESCRIPTION OF METHOD.

Weigh 2.425 grams of sample into a beaker, add 2 grams of carbonate of lime and 25 cc. of water, and let stand an hour, stirring well three or four times. Filter into a graduated flask and wash with successive portions of water nearly boiling. Cool, make up to volume, and evaporate an aliquot in a porcelain dish. Add a mixture of 1 cc. of strong nitric acid and 4 cc. of strong hydrochloric acid; again evaporate to dryness. Take up in hot water. Add a few drops of strong hydrochloric acid and platinum chloride solution and evaporate as usual. Wash the precipitate first with acid alcohol (10 cc. of strong hydrochloric acid to 100 cc. of 95 per cent alcohol), then complete as usual. If 50 cc. is used (0.485 gram) multiply the weight of the double salt by 40 to get the percentage of potassium oxide.

#### TEST OF THE METHOD.

The table contains some tests of the method on known mixtures made by S. E. Asbury. Blanks were run on all the ingredients used. It was hoped that some mixtures that absorbed potash by the regular method would be available, but absorption did not occur.

*Potash by official and by new method compared with calculated composition.*

LABORATORY NO.	OFFICIAL	NEW METHOD	CALCULATED
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
22538	5.63	5.58	5.45
22539	6.12	6.13	6.05
22540	6.57	6.91	6.57
22541	2.29	2.29	2.29
22542	2.99	2.98	3.04
22543	3.60	3.68	3.68
22544	2.49	2.57	2.74
22545	3.82	3.58	3.61
22546	4.11	4.37	4.35
Average	4.18	4.23	4.20

### REPORT ON INORGANIC PLANT CONSTITUENTS.

Determination of Iron and Aluminium, Calcium and Magnesium.

By A. J. PATTEN (Agricultural Experiment Station,  
East Lansing, Michigan), *Referee*.

No cooperative work has been called for during the past two years. The referee has, however, devoted considerable time to a study of the methods<sup>1</sup> as last presented to the association, and slight changes have been incorporated.

<sup>1</sup> *This Journal*, 1923, 6: 421.



These methods have been of great service in the laboratory at this station in connection with certain investigations that required the analysis of plant material for the mineral elements. A large number of determinations have been made, and satisfactory results obtained for iron and aluminium with as little as 1.182 grams of air-dry material.

Jones and Pember of the Rhode Island Agricultural Experiment Station have also used the methods in an investigation of the fertilizer nutrients required by barley, wheat, and oats<sup>1</sup>.

The methods as now used in this laboratory are as follows:

#### IRON AND ALUMINIUM PHOSPHATES.

Weigh a quantity of material sufficient to yield about 0.5 gram of ash into a flat-bottomed platinum dish and ignite as directed in the official methods<sup>2</sup>. After removal of sand and silica reduce the solution to a volume of about 50 cc. Add a few drops of concentrated nitric acid and heat to boiling to oxidize any ferrous iron that may be present. Cool; add 0.5 gram of di-ammonium hydrogen phosphate and a few drops of thymol blue indicator (acid range). (When working with seeds or other materials containing a high percentage of phosphorus the addition of di-ammonium phosphate is unnecessary.) Exactly neutralize with dilute ammonium hydroxide (1 + 9) and add 25 cc. of a 25 per cent ammonium acetate solution. Let stand at room temperature until the precipitate has settled, filter, and wash 8-10 times with hot 5 per cent ammonium nitrate solution. Ignite and weigh as ferric and aluminium phosphate.

For the separation of iron and aluminium, the method as given under Waters, Brine, and Salt<sup>3</sup> is recommended.

No changes have been made in the methods for calcium and magnesium<sup>4</sup>.

### REPORT ON SULFUR AND PHOSPHORUS IN THE SEED OF PLANTS.

By W. L. LATSHAW<sup>5</sup> (Agricultural Experiment Station, Manhattan, Kans.), *Associate Referee*.

The object of the work for 1925 like that of 1924 was to secure additional information as to the reliability of the magnesium nitrate method for determining sulfur and phosphorus in the seed of plants. Samples of cottonseed meal, soy bean meal, and mustard seed meal, representing a portion of the samples used in past year's work<sup>6</sup>, were used for analyses.

<sup>1</sup> *Soil Science*, 1925, 19: 169.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 39, par. 2.

<sup>3</sup> *Ibid.*, 95, par. 57.

<sup>4</sup> For report of Sub-committee A and action of the association, see *This Journal*, 1926, 9: 75.

<sup>5</sup> Presented by A. J. Patten.

<sup>6</sup> *This Journal*, 1923, 6: 414; 1925, 8: 469.

The reagents and details of oxidation and solution have been published<sup>1</sup>. In regard to reagents it is of interest to note that it is now possible to secure a very high grade of magnesium nitrate crystals, practically sulfur-free. The use of the magnesium nitrate crystals obviates the necessity of making the chemical from calcined magnesia. The only precaution suggested is that an equivalent quantity of chemical be used<sup>1</sup>.

The collaborative results on the determination of sulfur and phosphorus given in the table were obtained by using the magnesium nitrate method<sup>2</sup>.

COLLABORATOR	MATERIAL	SULFUR	PHOSPHORUS
		<i>per cent</i>	<i>per cent</i>
R. W. Titus Manhattan, Kans. (Sample No. 3)	Cottonseed meal	0.54	0.92
		0.55	0.90
		0.52	
	Soy bean meal	0.42	0.52
		0.40	0.52
		0.42	
	Mustard seed meal	0.92	1.11
		0.93	1.13
		0.91	
J. F. Merrill Manhattan, Kans. (Sample No. 4)	Cottonseed meal	0.52	0.91
		0.52	0.92
	Soy bean meal	0.41	0.54
		0.40	0.52
	Mustard seed meal	0.89	1.15
		0.89	1.14
George Cooksey Student—Kansas State Agricultural College Manhattan, Kans. (Sample No. 3)	Cottonseed meal	0.51	0.91
		0.51	0.92
	Soy bean meal	0.39	0.52
		0.40	0.52
	Mustard seed meal	0.90	1.13
		0.91	1.14
W. L. Latshaw (Sample No. 5)	Cottonseed meal	0.50	0.91
		0.52	0.92
	Soy bean meal	0.40	0.53
		0.40	0.53
	Mustard seed meal	0.88	1.12
		0.91	1.12

<sup>1</sup> *This Journal*, 1923, 6: 414.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 45.

## DISCUSSION.

The results of this year's work are offered as further evidence in favor of the magnesium nitrate method for the determination of sulfur and phosphorus in plants and in the seed of plants.

The results are exceptionally uniform, the duplication is excellent, and when compared with the figures reported in 1923 and 1924 on an analysis from a portion of the same sample, the same uniformity is observed.

The size of charge and quantity of magnesium nitrate to be used are so important that it is considered advisable to repeat a word of caution offered in last year's report—that is, if more than a one gram sample is used, it is essential that a proportionally larger quantity of magnesium nitrate be used. The method as outlined is somewhat ambiguous, and if taken literally may lead to difficulty. The point of inference is that each gram of material to be oxidized requires 7.5 cc. of prescribed magnesium nitrate reagent or its equivalent in a less or greater dilution.

## CORRECTIONS.

Vol. IX, No. 1 (issue of February 15, 1926):

On page 32 the expression  $\text{Charge} \times \frac{200}{300} \times \frac{200}{250} \times \frac{50}{250}$  should read

$$\text{Charge} \times \frac{200}{500} \times \frac{200}{250} \times \frac{50}{250}.$$

On page 38 the expression  $\frac{2}{t^2 - 25}$  in parentheses, should read  $\frac{t^2 - 25}{2}$ .

## CONTRIBUTED PAPERS.

### COMPARISON OF THE COMMERCIAL GRADING OF BARLEY WITH THE MACROSCOPICAL AND CHEMICAL ANALYSIS.

By LLOYD C. MITCHELL (St. Louis Station, Bureau of Chemistry, U. S. Department of Agriculture).

This paper is devoted to a comparison of the commercial grading, the macroscopical examination, and the chemical analysis (moisture, ether extract, crude fiber, and nitrogen) of eighty samples of barley grown in Minnesota, Montana, and North and South Dakota during the year 1920. The chemical analysis is also given for the corresponding cleaned, hand-picked samples, and in this way the effect of extraneous materials on the composition of pure barley is shown. Further, the results for moisture and crude fiber on six cleaned, hand-picked samples of wild oats, the most common foreign material, are included.

#### SOURCE AND GRADES.

The samples of barley were collected and graded by the Minnesota State Inspection Office, Minneapolis, Minnesota, in its regular course of business during October and November 1920, on inbound shipments. The grades effective at that time were as follows:

#### GRADES FOR BARLEY<sup>1</sup>.

*No. 1 Barley.*—Shall be sound, plump, bright, cool, and sweet; shall weigh not less than 48 pounds to the measured bushel; and shall contain not more than:

- 1 per cent of dirt or weed seeds combined,
- 2 per cent of other grains, which may include not more than 1 per cent of wild oats.

*No. 2 Barley.*—Shall be sound, cool, and sweet, and of healthy color; shall weigh no less than 46 pounds to the measured bushel; and shall contain not more than:

- 2 per cent of dirt or weed seeds combined,
- 5 per cent of other grains, which may include not more than 3 per cent of wild oats.

*No. 3 Barley.*—Shall be cool, sweet, and reasonably sound; shall weigh not less than 44 pounds to the measured bushel; and shall contain not more than:

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<sup>1</sup> Minnesota grades—effective August 30, 1920.

- 4 per cent of dirt and weed seeds combined,
- 7 per cent of other grains, which may include not more than
- 5 per cent of wild oats.

*No. 4 Barley.*—Shall be cool, but may be slightly damaged; shall weigh not less than 41 pounds to the measured bushel; and shall contain not more than:

- 6 per cent of dirt or weed seeds combined,
- 10 per cent of other grains, which may include not more than
- 8 per cent of wild oats.

*Sample Grade.*—All barley that does not come within the requirements of the above grades, or that is not safe for warehousing, or for any other reason is unfit for any of the above grades shall be classed "sample grade", with inspector's notation as to quality and condition.

#### METHOD OF GRADING.

It will be noted that barley is graded on its physical characteristics such as soundness, maturity, color, odor, condition, weight, and the presence or absence of extraneous material, such as other grains, chaff and dirt, and weed seeds, particularly wild oats.

A representative sample was secured by authorized inspectors of the Minnesota State Inspection Office, by inserting a grain trier in various places throughout the entire carload of barley. The sample thus taken was thoroughly mixed, and the weight per bushel was found by means of a Winchester test-kettle. The percentage of weed seeds and chaff and dirt was then determined by passing a known quantity of the sample through a buckwheat sieve having triangular perforations (side of perforations  $\frac{1}{8}$  inch long). Finally, the percentages of foreign grains and wild oats were determined by separation by means of handpicking. The results of the grading are given in Table 1.

It will be observed from Table 1 that the samples of barley were graded largely by the test weight per bushel. From Tables 1 and 4 it will be noted that the weight per bushel depended more or less on the quantity and kind of foreign material present and on the plumpness (weight) of the barley kernels.

The origin of the samples by States is shown in Table 2.

From Table 3 it will be seen that the four States from which the samples originated produced approximately 40 per cent of the barley crop grown in the United States in 1920.

#### PREPARATION OF SAMPLES.

In order to arrive at the physical composition of the product and to ascertain the effect of the extraneous material on its composition, two

portions from each of the eighty thoroughly mixed samples of barley were removed by the writer for analysis. The first portion, consisting of 100 grams, was handpicked. After noting the percentages of the various grains, weed seeds, etc., which are given in detail in Table 4 and summarized in Table 5, the portions thus separated were remixed and designated in Table 6 as "field run". From the second portion, consisting of approximately 100 grams, all the foreign materials were removed by handpicking and discarded. The barley remaining is designated in Table 6 as "pure". Each of the subdivisions thus handpicked was ground to pass a 40-mesh sieve and analyzed separately in accordance with the official methods<sup>1</sup>. The results of analysis are found in Table 6.

The crude fiber results for barley, as given in Table 6, are summarized in Table 7. In Table 8 are given the results for moisture and crude fiber on six samples<sup>2</sup> of cleaned, handpicked wild oats, separated from the so-called mill-oats of commerce.

#### DISCUSSION AND SUMMARY.

In the grading of barley, the definitions for the four grades permit wild oats to be classified under the caption "other grains", or more frequently as a certain percentage of "wild oats and other grains". Wild oats are usually determined as "sample grade" barley, which was defined previously. When allowances are made for these conditions, the grading and macroscopical examination agree reasonably well.

In general, the foreign grains and weed seeds occurring naturally in barley do not materially affect the moisture, ether extract, or nitrogen content, but in practically every instance they do cause an increase in the fiber content.

While the average weight per bushel and per hundred kernels decreases and the crude fiber increases from the higher to the lower grades, there appears to be no definite ratio among these values for the individual samples. Usually, the higher the fiber the more foreign or extraneous material is present.

Those samples of pure barley showing the higher crude fiber values apparently contain considerable amounts of immature barley, the so-called barley needles, as is shown by the weight per hundred kernels.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925.

<sup>2</sup> Prepared and analyzed by O. S. Keener.

TABLE 1.  
*Classification of the barley samples by grades\*.*

SAMPLE NUMBER	TEST WEIGHT PER BUSHEL	WILD OATS	DIRT	WEED SEEDS	REMARKS
	<i>pounds</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
GRADE NO. 2.					
1	47.0	3.5			
2	46.0	1.0			
3	47.0	1.0			1 per cent other grains.
4	47.0	2.0			
5	46.0				
6	48.0				3 per cent wild oats and dirt.
7	49.0				3 per cent other grains.
8	47.0		2.0		
9	46.0	1.0		1.0	
10	47.0		2.0		
Maximum	49.0				
Minimum	46.0				
Average	47.0				
GRADE NO. 3.					
11	44.0				
12	44.5	5.0			
13	45.5				
14	44.5			2.0	7 per cent wild oats and other grains.
15	47.0				6 per cent wheat.
16	44.0	3.0		2.0	
17	46.0				5 per cent wild oats and other grains.
18	44.5		2.0		7 per cent other grains.
19	44.5				5 per cent wild oats and other grains.
20	45.5				7 per cent wild oats and other grains.
21	44.5	5.0			
22	45.5	2.0			
23	47.5	4.0			
24	45.0	2.0	3.0		
25	45.0				4 per cent wild oats and other grains.
26	44.5	5.0			
27	45.0	3.0	3.0		
28	45.0	5.0		3.5	Stained.
29	45.5	2.0		1.0	
30	45.5	2.0		1.0	
Maximum	47.5				
Minimum	44.0				
Average	45.15				
GRADE NO. 4.					
31	42.5		2.0		
32	41.0		5.5		
33	43.0	8.0		5.0	6 per cent wild oats and other grains.
34	42.0	5.0		2.0	
35	41.0	8.0	1.0		
36	43.0				4 per cent wild oats and other grains.
37	44.0			1.0	6 per cent wild oats and other grains.
38	42.0	5.0			
39	46.0	8.0	1.0		
40	41.5	8.0		2.0	

TABLE 1—Continued.

SAMPLE NUMBER	TEST WEIGHT PER BUSHEL	WILD OATS	DIRT	WEED SEEDS	REMARKS
	<i>pounds</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
41	42.0				8 per cent other grains.
42	42.5				3 per cent other grains.
43	41.0	4.0			
44	41.0	3.0			
45	43.0				7 per cent wild oats and other grains.
46	43.5	5.0	3.0		2 per cent foreign matter.
47	42.0	3.0	3.5		
48	46.0				10 per cent wild oats and other grains.
49	42.0	5.0		2.5	
50	47.0	8.0	1.0		
Maximum	47.0				
Minimum	41.0				
Average	42.8				
SAMPLE GRADE.					
51	44.5	1.0	7.0		
52	41.0	11.0			
53	36.0	25.0			
54	41.5	12.0			
55	38.5	18.0			
56	41.0	1.0	6.0		
57	41.0	18.0		1.0	
58	48.0	9.0			10 per cent dirt and flaxseed.
59	40.0	3.0			
60	42.5	10.0			
61	39.0				7 per cent other grains.
62	44.0	11.0			
63	45.0	12.0		2.0	
64	43.5	17.0	2.5		
65	37.5	12.0			Musty.
66	39.0	10.0		3.0	
67	42.5		4.0		20 per cent wild oats and other grains.
68	40.0	5.0			3 per cent foreign matter.
69	39.5			4.0	5 per cent wild oats and other grains.
70	39.0	7.0			
71	38.5				
72	40.5	6.0			
73	37.0				15 per cent wild oats and other grains.
74	42.0	15.0	5.0		
75	46.0	11.0		4.0	
76	44.0		7.0		
77	41.0	17.0		3.0	Stained.
78	45.0	8.0		5.0	10 per cent wheat.
79	39.0	10.0			2 per cent foreign matter.
80	42.0			9.0	
Maximum	48.0				
Minimum	36.0				
Average	41.3				

\* As graded by the Minnesota State Inspection Office, Minneapolis, Minn.



TABLE 2.  
*Origin of barley samples.*

MINNESOTA INSPECTION GRADE	STATE WHERE GROWN					
	Minnesota	Montana	North Dakota	South Dakota	Unknown	Total
No. 2	3	0	1	6	0	10
No. 3	3	0	4	13	0	20
No. 4	8	0	4	8	0	20
Sample	8	1	10	9	2	30
Total	22	1	19	36	2	80

TABLE 3.  
*Production data for the year 1920 in bushels\*.*

California.....	28,750,000	Montana.....	1,152,000
Illinois.....	5,533,000	Nebraska.....	7,424,000
Iowa.....	4,950,000	North Dakota.....	19,530,000
Kansas.....	19,482,000	South Dakota.....	25,700,000
Michigan.....	6,630,000	Wisconsin.....	15,913,000
Minnesota.....	22,375,000	All other States.....	31,893,000
Total for United States.....			189,332,000
Total for Minnesota, Montana, and Dakotas.....			68,757,000

\* U. S. Dept. Agr. Yearbook, 1921.

TABLE 4.

*Physical composition of the "field run" barley.*

SAMPLE NUMBER	BARLEY	OTHER GRAINS					WEED SEEDS, ETC.				
		Wheat	Oats	Rye	Flax	Vetch	Wild Oats	Wild Buck- wheat	Mus- tard	Other Weed Seeds, Dirt*	Straw, Stems
		per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
GRADE NO. 2.											
1	94.19	0.26	0.56				2.38	0.39		1.72	0.50
2	97.17	0.40	0.11				1.70	0.90		0.48	0.05
3	93.57	0.93	0.32	1.87			2.45	0.28		0.50	0.08
4	94.97	0.44	1.14				0.17	1.37	0.39	1.47	0.05
5	97.34	0.68	0.24	0.45			0.17			1.12	
6	94.99	0.40	1.14				1.83	0.93		0.65	0.06
7	93.97	0.19	0.39				4.00	0.10		1.22	0.13
8	94.15	0.29	2.27		0.92		0.08	0.46		1.78	0.05
9	92.81	1.47	1.58				1.10	0.37		2.67	
10	96.03	0.98	0.19		0.27		1.66			0.87	
Maximum	97.34	1.47	2.27				4.00	1.37		2.67	0.50
Minimum	92.81	0.19	0.11				0.08			0.48	
Average	94.92	0.60	0.79				1.55			1.25	
GRADE NO. 3.											
11	91.80	0.99	0.94				3.52	0.13		2.47	0.15
12	94.30	0.58	0.48				3.29	0.35	0.20	0.70	0.10
13	95.59	0.22	0.73				1.72	0.16		1.48	0.10
14	94.12	0.22	0.86				1.68	0.59		2.13	0.40
15	87.69	9.43	0.27		0.18		0.49	0.10		1.72	0.12
16	91.53	1.71	0.17				3.72	0.35		2.40	0.12
17	97.15	0.25	0.20		0.05		1.55	0.09		0.67	0.04
18	87.93	3.90	1.10				3.84	0.25	0.65	2.36	0.07
19	93.35	0.21	0.75	0.45			2.76	0.92	0.25	1.20	0.11
20	93.02	0.35	0.32				5.40	0.15		0.69	0.07
21	90.71	0.24	2.89				4.23	0.46		1.31	0.16
22	94.31	0.40	2.37				0.64	0.83		1.31	0.14
23	92.42	0.44	0.79	1.23			2.74	0.49		1.74	0.15
24	91.97	2.98	0.46				1.97	0.20		2.22	0.20
25	94.28	0.83	2.57				0.76			2.99	0.57
26	90.17	0.70	0.51				6.12	0.21		2.19	0.10
27	91.44	0.62	0.76				2.32	1.20	0.44	3.04	0.18
28	91.82	0.50	1.91				3.11	0.87	0.18	1.49	0.12
29	87.35	4.78	2.01				3.22	0.24		2.40	
30	93.48	0.58	1.59				1.31			2.91	0.13
Maximum	97.15	9.43	2.89				6.12	1.20		3.04	0.57
Minimum	87.35	0.21	0.17				0.49	0.00		0.70	0.00
Average	92.22	1.50	1.08				2.72			1.87	0.15
GRADE NO. 4.											
31	94.43	0.35	0.06				0.07	0.33		4.34	0.12
32	86.93	0.91	0.75				6.69	0.37		4.25	0.10
33	91.17		1.65			0.10	4.33	0.13	0.27	2.20	0.15
34	92.53		1.28				2.85	0.34	0.48	2.12	0.40
35	90.10	0.07	0.71				5.91	0.05	1.45	1.59	0.11
36	91.88		5.51				1.62			0.77	0.22
37	91.01	0.07	1.93				4.26	0.69		1.89	0.15
38	91.04		6.23				1.53			0.75	0.45
39	92.92	0.14	0.41			0.16	5.55	0.09		0.68	0.05

TABLE 4—Continued.

SAMPLE NUMBER	BARLEY	OTHER GRAINS					WEED SEEDS, ETC.				
		Wheat	Oats	Rye	Flax	Vetch	Wild Oats	Wild Buck- wheat	Must- ard	Other Weed Seeds, Dirt*	Straw, Stems
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
40	88.61	0.50	2.17	0.08			5.18		1.79	1.60	0.15
41	88.39		7.68				0.37	0.63	0.17	2.54	0.22
42	92.76	0.08	3.55				0.80	0.78		1.84	0.11
43	91.46		3.40				2.98	0.69	0.22	1.07	0.18
44	89.28	0.44	4.40				3.37	0.20	0.09	1.99	0.23
45	84.64	0.29	7.69				3.85	0.68		2.85	
46	86.56	0.41	1.42				5.75	0.27	2.00	3.45	0.14
47	85.54		9.40				2.80		0.54	1.72	
48	87.90	3.51	4.04				3.10	0.40		1.00	0.05
49†	79.68	0.92	1.53				14.46	0.35		2.87	0.19
50	88.10	0.14	0.26				9.57	0.46		1.32	0.15
Maximum	94.43	3.51	9.40				9.57	0.46	2.00	4.34	0.45
Minimum	84.64		0.06				0.07			0.68	
Average	89.75		3.29				3.71			2.00	
SAMPLE GRADE.											
51	90.79	0.68	0.52	1.38			1.26	2.05	0.20	3.75	0.67
52	83.48	0.16	0.85				12.54	0.33		2.56	0.08
53	71.37	0.62	0.39				25.19	0.08	0.60	1.59	0.16
54	83.29	0.54	1.11				12.01	0.10	0.45	1.11	0.10
55	83.36	0.35	1.72				12.89	0.44		1.12	0.12
56	78.55	0.32	1.85				10.72	0.37	4.37	3.49	0.33
57	76.17	0.20	1.74			0.12	17.60	0.19		3.83	0.15
58	79.37	0.35	0.12		8.90	0.25	6.50	0.13		4.20	0.18
59	91.78		3.22				3.10	0.07	0.31	1.39	0.13
60	88.54	0.42	0.66		0.07		9.00	0.14	0.13	0.55	0.08
61	84.97	0.86	0.42	0.41			9.19	0.14	1.99	2.17	0.26
62	88.39	0.15	0.40			0.25	9.74	0.11	0.11	0.85	0.10
63	78.01	4.20	0.28			0.15	12.80	0.22	0.32	3.90	0.14
64	76.99	0.29	0.59				19.44			2.44	0.25
65	86.86	0.10	0.22				10.02		0.28	2.39	0.13
66	71.63	1.80	5.45				17.81	0.09	0.77	2.28	0.17
67	80.65	0.48	0.33				15.36	0.25	1.84	0.98	0.11
68	92.93	0.45	1.53				2.62	0.14		2.16	0.17
69	88.44		2.76				2.23	0.31		6.03	0.23
70	85.55	0.71	1.39				5.79	0.10	1.27	4.99	0.20
71	88.67		4.80	3.62			4.08	0.22		1.97	0.26
72	90.85	0.17	0.69				3.47		0.62	4.20	
73	84.32	0.30	4.18				7.46	0.30		2.77	0.67
74	77.14	0.74	2.52				11.00	0.21	1.36	3.13	0.28
75	86.65	0.74	0.27				7.36	0.85	2.48	1.51	0.14
76	86.47	0.36	0.49				3.74	0.34	2.49	5.88	0.23
77	81.87	0.52	0.34				14.02	0.31	0.78	2.02	0.14
78	74.07	8.45	1.35				7.37	0.65		6.78	0.15
79	78.19	2.11	0.67				15.83	0.28	0.97	1.67	0.30
80	79.38	0.97	1.68				10.89	0.59	2.92	3.43	0.14
Maximum	92.93	8.45	5.45				25.19	2.05	4.37	6.78	0.67
Minimum	71.37	0.00	0.12				1.26	0.00		0.55	0.00
Average	82.96	1.00	1.42				10.03	0.33		2.84	0.20

\* Large green and yellow foxtail and some chaff.

† Not included in maximum, minimum, or average.

TABLE 5.

*Physical composition of the "field run" barley—summary.*

SAMPLE NUMBER	TEST WEIGHT PER BUSHEL	WEIGHT 100 KERNELS BARLEY	Barley	FOREIGN MATERIALS		
				Total	Other Grains	Weed seeds, Chaff, Straw, Stems, dirt, etc.
	<i>pounds</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
GRADE NO. 2.						
1	47.0	2.85	94.19	5.81	0.81	4.99
2	46.0	3.00	97.17	2.83	0.51	2.32
3	47.0	3.10	93.57	6.43	3.12	3.31
4	47.0	2.88	94.97	5.03	1.58	3.45
5	46.0	2.84	97.34	2.66	1.37	1.29
6	48.0	3.13	94.99	5.01	1.54	3.47
7	49.0	2.88	93.97	6.03	0.58	5.45
8	47.0	2.93	94.15	5.85	3.48	2.37
9	46.0	2.80	92.81	7.19	3.05	4.14
10	47.0	3.04	96.03	3.97	1.44	2.53
Maximum	49.0	3.13	97.34	7.19	3.48	5.45
Minimum	46.0	2.80	92.81	2.66	0.51	1.29
Average	47.0	2.95	94.92	5.08	1.75	3.33
GRADE NO. 3.						
11	44.0	2.69	91.80	8.20	1.93	6.27
12	44.5	2.50	94.30	5.70	1.06	4.64
13	45.5	2.83	95.59	4.41	0.95	3.46
14	44.5	2.90	94.12	5.88	1.08	4.80
15	47.0	3.14	87.69	12.31	9.88	2.43
16	44.0	2.85	91.53	8.47	1.88	6.59
17	46.0	3.13	97.15	2.85	0.50	2.35
18	44.5	2.73	87.93	12.07	5.00	7.07
19	44.5	3.04	93.35	6.65	1.41	5.24
20	45.5	2.85	93.02	6.98	0.67	6.31
21	44.5	2.89	90.71	9.29	3.13	6.16
22	45.5	3.00	94.31	5.69	2.77	2.92
23	47.5	3.01	92.42	7.58	2.46	5.12
24	45.0	3.14	91.97	8.03	3.44	4.59
25	45.0	2.66	94.28	5.72	3.40	2.32
26	44.5	2.93	90.17	9.83	1.21	8.62
27	45.0	3.19	91.44	8.56	1.38	7.18
28	45.0	2.97	91.82	8.18	2.41	5.77
29	45.5	2.75	87.35	12.65	6.79	5.86
30	45.5	2.96	93.48	6.52	2.17	4.35
Maximum	47.5	3.19	97.15	12.65	9.88	8.62
Minimum	44.0	2.50	87.35	2.85	0.50	2.35
Average	45.15	2.91	92.22	7.78	2.68	5.10
GRADE NO. 4.						
31	42.5	2.91	94.43	5.57	0.71	4.86
32	41.0	2.45	86.93	13.07	1.66	11.41
33	43.0	2.91	91.17	8.83	1.75	7.08
34	42.0	2.87	92.53	7.47	1.28	6.19
35	41.0	2.81	90.10	9.90	0.78	9.12
36	43.0	2.86	91.88	8.12	5.51	2.61
37	44.0	2.70	91.01	8.99	2.00	6.99
38	42.0	2.87	91.04	8.96	6.23	2.73

TABLE 5—Continued.

SAMPLE NUMBER	TEST WEIGHT PER BUSHEL	WEIGHT 100 KERNELS BARLEY	Barley	FOREIGN MATERIALS		
				Total	Other Grains	Weed seeds, Chaff, Straw, Stems, dirt, etc.
	<i>pounds</i>	<i>grams</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
39	46.0	2.99	92.92	7.08	0.71	6.37
40	41.5	2.77	88.61	11.39	2.67	8.72
41	42.0	2.97	88.39	11.61	7.68	3.93
42	42.5	2.92	92.76	7.24	3.71	3.53
43	41.0	2.93	91.46	8.54	3.40	5.14
44	41.0	2.89	89.28	10.72	4.84	5.88
45	43.0	2.90	84.64	15.36	7.98	7.38
46	43.5	2.97	86.56	13.44	1.83	11.61
47	42.0	2.92	85.54	14.46	9.40	5.06
48	46.0	3.23	87.90	12.10	7.55	4.55
49*	42.0	2.85	79.68	20.32	2.45	17.87
50	47.0	2.89	88.10	11.90	0.40	11.50
Maximum	47.0	3.23	94.43	15.36	9.40	11.61
Minimum	41.0	2.45	84.64	5.57	0.71	2.61
Average	42.8	2.88	89.75	10.25	3.69	6.56
SAMPLE GRADE.						
51	44.5	2.73	90.79	9.21	1.28	7.93
52	41.0	2.72	83.48	16.52	1.01	15.51
53	36.0	2.80	71.37	28.63	1.01	27.62
54	41.5	2.88	83.29	16.71	2.94	13.77
55	38.5	2.59	83.36	16.64	2.07	14.57
56	41.0	2.85	78.55	21.45	2.17	19.28
57	41.0	2.60	76.17	23.83	2.06	21.72
58	48.0	2.84	79.37	20.63	9.62	11.01
59	40.0	2.33	91.78	8.22	3.22	5.00
60	42.5	2.85	88.54	11.46	1.56	9.90
61	39.0	2.24	84.97	15.03	1.28	13.75
62	44.0	2.90	88.39	11.61	0.70	10.91
63	45.0	2.90	78.01	21.99	4.63	17.36
64	43.5	3.18	76.99	23.01	0.88	22.13
65	37.5	2.31	86.86	13.14	0.32	12.82
66	39.0	2.64	71.63	28.37	7.25	21.12
67	42.5	2.90	80.65	19.35	0.81	18.54
68	40.0	2.58	92.93	7.07	1.98	5.09
69	39.5	2.40	88.44	11.56	2.76	8.80
70	39.0	2.54	85.55	14.45	2.10	12.35
71	38.5	2.53	88.67	11.33	4.80	6.53
72	40.5	2.47	90.85	9.15	0.86	8.29
73	37.0	2.55	84.32	15.68	4.48	11.20
74	42.0	2.88	77.14	22.86	6.88	15.98
75	46.0	3.17	86.65	13.35	1.01	12.34
76	44.0	2.87	86.47	13.53	0.85	12.68
77	41.0	2.89	81.87	18.13	0.86	17.27
78	45.0	2.76	74.07	25.93	10.98	14.95
79	39.0	2.87	78.19	21.81	2.78	19.03
80	42.0	3.02	79.38	20.62	2.65	17.97
Maximum	48.0	3.18	92.93	28.63	10.98	27.62
Minimum	36.0	2.24	71.37	7.07	0.32	5.09
Average	41.3	2.73	82.96	17.04	2.86	14.18

\* Not included in maximum, minimum, or average.

TABLE 6.  
*Chemical analysis of barley.*

SAMPLE NUMBER	FIELD RUN				PURE			
	Mois- ture	Ether Extract	Crude Fiber	Nitro- gen	Mois- ture	Ether Extract	Crude Fiber	Nitro- gen
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
GRADE NO. 2.								
1	9.25	1.50	5.08	1.91	9.12	1.24	5.02	1.89
2	9.41	1.75	5.26	2.03	9.71	1.38	4.85	2.07
3	8.75	1.47	5.24	1.86	8.37	1.94	5.14	1.81
4	8.40	2.34	5.52	1.88	8.25	2.02	5.23	1.91
5	8.44	1.95	5.19	1.89	8.47	1.95	5.00	1.95
6	8.66	2.30	5.56	1.82	8.27	2.18	5.20	1.81
7	8.60	1.69	4.95	1.91	8.79	1.43	4.27	1.92
8	9.39	1.60	4.88	1.99	8.81	1.58	4.92	2.00
9	8.44	1.84	5.54	1.91	8.58	1.55	5.38	1.88
10	9.83	1.45	5.38	1.81	9.73	1.65	5.23	1.75
Maximum	9.83	2.34	5.56	2.03	9.73	2.18	5.38	2.07
Minimum	8.40	1.45	4.88	1.81	8.25	1.24	4.27	1.75
Average	8.92	1.79	5.26	1.90	8.81	1.69	5.02	1.90
GRADE NO. 3.								
11	8.87	1.88	5.94	1.93	9.01	1.62	4.67	1.94
12	9.91	1.85	5.79	1.82	9.06	1.85	5.23	1.89
13	9.05	1.50	5.65	2.09	8.38	1.41	5.05	2.14
14	9.66	1.32	5.44	2.00	8.43	1.39	5.13	2.07
15	8.38	1.88	5.37	1.99	9.23	1.43	5.27	1.87
16	8.02	1.81	6.41	1.84	7.72	1.91	5.87	1.86
17	8.67	1.84	5.54	1.92	8.57	1.65	5.49	1.92
18	9.36	1.85	5.90	2.12	8.20	1.85	5.10	2.13
19	8.60	1.85	6.05	1.70	8.04	1.58	5.49	1.70
20	8.15	1.66	5.46	1.82	8.01	1.54	5.38	1.79
21	8.11	2.01	5.86	2.09	8.99	1.84	4.85	2.07
22	8.11	1.95	5.59	1.88	8.54	1.83	5.09	1.91
23	8.52	2.05	5.35	1.86	8.32	2.06	4.62	1.93
24	8.98	2.01	5.47	1.70	8.53	1.88	5.39	1.70
25	8.63	1.55	6.07	1.86	8.34	1.57	5.33	1.89
26	9.10	1.82	6.20	1.99	9.08	1.53	5.14	1.98
27	8.35	2.11	5.91	1.89	8.25	1.85	5.43	1.86
28	8.61	1.91	5.71	1.92	9.46	1.64	5.63	1.91
29	8.43	2.09	5.82	1.95	8.20	1.84	5.37	1.89
30	8.83	1.78	6.14	1.85	8.23	1.92	5.74	1.82
Maximum	9.91	2.11	6.41	2.12	9.41	2.06	5.87	2.14
Minimum	8.02	1.32	5.35	1.70	7.72	1.39	4.62	1.70
Average	8.72	1.84	5.78	1.91	8.53	1.71	5.26	1.91
GRADE NO. 4.								
31	9.07	1.22	5.94	1.95	8.85	....	5.53	2.05
32	8.61	1.15	6.47	1.81	8.42	....	5.21	1.93
33	9.96	1.16	6.14	1.79	8.51	....	5.35	1.79
34	10.21	1.01	6.32	1.77	9.18	...	5.62	1.77
35	8.76	1.50	6.56	2.00	8.53	....	5.48	2.05
36	8.30	1.12	6.14	2.13	8.07	1.02	5.71	2.07
37	9.65	1.38	5.64	1.85	9.81	....	5.30	1.81
38	8.37	1.31	6.52	2.00	8.57	1.30	5.82	2.02
39	9.36	0.97	5.46	1.95	9.28	1.05	4.98	2.02
40	9.70	1.51	6.70	2.05	9.29	1.11	5.65	2.03
41	8.50	1.38	6.21	1.85	8.90	1.21	5.94	1.82

TABLE 6—Continued.

SAMPLE NUMBER	FIELD RUN				PURE			
	Mois- ture	Ether Extract	Crude Fiber	Nitro- gen	Mois- ture	Ether Extract	Crude Fiber	Nitro- gen
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
42	8.21	1.33	6.06	1.85	8.90	1.34	5.70	1.86
43	8.71	1.50	5.77	1.72	8.35	1.29	5.39	1.81
44	9.28	1.74	6.66	1.93	8.61	1.55	5.57	2.00
45	8.73	1.74	6.60	1.78	9.26	1.30	5.59	1.72
46	7.88	2.08	6.44	1.98	8.67	1.58	5.56	1.99
47	8.30	2.11	6.43	1.96	8.50	1.62	5.69	1.95
48	9.13	1.84	5.69	1.85	8.79	1.80	5.44	1.82
49*	9.15	1.60	7.35	1.70	9.02	1.23	5.04	1.79
50	8.36	1.60	5.83	1.89	8.75	1.32	4.69	1.86
Maximum	10.21	2.11	6.70	2.13	9.81	1.80	5.94	2.07
Minimum	7.88	0.97	5.46	1.72	8.07	1.02	4.69	1.72
Average	8.90	1.45	6.13	1.90	8.81	1.34	5.46	1.91
SAMPLE GRADE.								
51	8.73	1.92	6.11	2.07	9.22	1.22	5.69	2.07
52	7.95	1.70	7.16	1.68	9.09	1.47	5.95	1.78
53	7.35	2.82	8.65	1.70	9.28	1.54	5.87	1.68
54	7.73	2.22	7.18	1.91	8.10	1.72	5.00	1.91
55	8.00	1.99	7.32	1.93	8.46	1.34	5.64	2.00
56	7.44	2.78	7.23	2.00	8.61	1.41	5.34	1.93
57	7.47	2.78	7.03	1.84	7.96	1.63	5.64	1.77
58	7.34	2.57	5.87	1.85	8.06	1.44	4.64	1.64
59	7.87	2.00	7.05	2.16	7.97	1.56	6.42	2.05
60	8.28	2.07	6.01	1.86	8.13	1.57	5.24	1.82
61	8.41	2.55	7.94	1.75	8.38	2.12	6.70	1.75
62	9.32	1.77	6.04	1.92	8.78	1.56	5.28	1.89
63	8.11	2.46	6.48	1.83	8.72	1.50	5.03	1.77
64	8.21	2.44	6.97	1.60	8.13	1.66	5.27	1.64
65	8.57	1.94	7.47	1.89	8.16	1.41	6.18	1.84
66	8.51	2.65	7.50	2.16	8.77	1.70	5.83	1.92
67	8.42	1.74	6.25	1.96	8.77	1.66	4.81	1.96
68	8.94	1.07	6.21	1.93	9.05	1.02	5.87	1.92
69	9.35	1.33	6.51	1.99	9.21	1.07	5.68	1.95
70	9.13	1.21	6.81	1.82	9.16	1.12	5.37	1.78
71	9.01	1.52	6.58	1.72	9.73	1.17	5.91	1.72
72	9.23	1.41	6.45	1.93	8.98	1.27	5.51	1.96
73	8.40	1.98	6.97	1.86	9.60	1.47	5.63	1.96
74	7.69	2.77	6.76	2.09	8.62	1.56	5.09	2.09
75	8.32	2.86	5.80	1.93	9.58	1.70	4.80	1.86
76	8.57	2.72	6.23	1.88	8.54	1.81	5.08	1.88
77	7.96	2.79	6.31	1.75	8.20	1.83	5.07	1.85
78	8.58	3.58	5.67	2.26	8.35	2.04	4.80	2.24
79	8.02	2.37	6.65	1.74	8.80	1.48	4.91	1.89
80	8.63	2.77	5.99	1.99	8.28	1.74	4.36	1.91
Maximum	9.35	3.58	8.65	2.26	9.73	2.12	6.70	2.24
Minimum	7.34	1.07	5.67	1.60	7.96	1.02	4.36	1.64
Average	8.65	2.23	6.71	1.90	8.69	1.52	5.42	1.88
PURE BARLEY (ALL SAMPLES).								
Maximum	....	....	....	....	9.81	2.18	6.70	2.24
Minimum	....	....	....	....	7.72	1.02	4.27	1.64
Average	....	....	....	....	8.70	1.61	5.34	1.90

\* Not included in maximum, minimum, or average.

TABLE 7.

*Crude fiber content of pure barley—summary.*

RANGE OF CRUDE FIBER IN PERCENTAGE	SAMPLES	
	Number	Per cent
4.25-4.49	2	2.50
4.50-4.74	4	5.00
4.75-4.99	8	10.00
5.00-5.24	20	25.00
5.25-5.49	18	22.50
5.50-5.74	17	21.25
5.75-5.99	8	10.00
6.00-6.24	1	1.25
6.25-6.49	1	1.25
6.50 and above	1	1.25

TABLE 8.

*Chemical analysis of wild oats\*.*

SAMPLE NUMBER	MOISTURE	CRUDE FIBER
	<i>per cent</i>	<i>per cent</i>
1	10.62	14.94
2	9.98	12.96
3	9.50	12.88
4	9.37	13.53
5	9.43	15.76
6	8.92	15.44

\* Analysis by O. S. Keener.

## THE ANALYSIS OF BUTTER.

By LLOYD C. MITCHELL and SAMUEL ALFEND (St. Louis Station<sup>1</sup>,  
Bureau of Chemistry, United States Department of Agriculture).

An Act of Congress<sup>2</sup>, approved March 4, 1923, provides "That for the purposes of the Food and Drugs Act of June 30, 1906, 'butter' shall be understood to mean the food product usually known as butter, \* \* \* and containing not less than 80 per centum by weight of milk fat, all tolerances having been allowed for".

By this Act butter gained the distinction of being the only food product for which a numerical standard is provided directly by Congress. Since the phrase "all tolerances having been allowed for" makes it incumbent upon food officials to take action against butter even very slightly deficient in milk fat, it behooves official chemists, in fairness to

<sup>1</sup> Ernest R. Smith, Chief.<sup>2</sup> Public—No. 519—67th Congress (H. R. 12053). An Act to define butter and to provide a standard therefor.



butter manufacturers, to employ accurate methods in determining milk fat.

The three steps in the examination of butter are (1) the sampling of the butter, (2) the preparation of the samples for analysis, and (3) the actual analysis of a small portion of the prepared sample.

Considerable work has been done on the sampling of butter, some of which has been published<sup>1</sup>. The writers have much information bearing upon this problem, but the results of their work have not yet been offered for publication. The preparation of the sample for analysis has been thoroughly studied by the writers, and the results have been published<sup>2</sup>.

The official methods<sup>3</sup> for the analysis of the prepared sample of butter are generally considered satisfactory, but in certain respects the writers have found them far from perfect. This paper presents some observations on the official methods for determining fat in butter, together with a proposed modification of the methods.

The usual analysis of butter includes the direct determination of moisture, salt, and curd, and the calculation of fat by difference. Although there is an official method for the direct determination of fat, the method is somewhat impracticable for ordinary routine work.

#### POSSIBILITIES OF ERROR IN OFFICIAL METHODS.

A study of the official methods revealed certain sources of possible error, *viz.*,

(a) *Failure to remove all the dried sample from the dish when transferring to a Gooch crucible.*—In general routine work, it is difficult to remove all the fat from the dish by means of the solvent. Pure dried butterfat was placed in evaporating dishes and washed out with petroleum ether. The quantity of butterfat retained in the dishes is shown in Table 1.

Failure to remove the fat only would not be harmful in the indirect method, but it is often very difficult to remove all the non-fat solids, particularly when lead or aluminum drying dishes are used. Too vigorous use of the rubber policeman should be avoided as it causes some of the rubber to come off and contaminate the solids.

(b) *Failure to remove all the fat from the asbestos in the filter crucible.*—It has been observed by the writers that the weight of the asbestos pads used for this purpose by different analysts varies from 0.2–2 grams or more. That this variation may involve an error due to failure of the

<sup>1</sup> *J. Dairy Sci.*, 1925, 8: 80.

<sup>2</sup> *This Journal*, 1925, 8: 574.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 276.

TABLE 1.  
*Butterfat retained in evaporating dish.*

SAMPLE NO.	BUTTERFAT LEFT IN DISH AFTER TRANSFER	CONDITION OF BUTTERFAT WHEN WASHED	SOLVENT	
			Quantity *	Temperature
	<i>grams</i>		<i>cc.</i>	<i>°C.</i>
1	0.0017	Solid	50	23
2	0.0100	Solid	50	33
3	0.0029	Melted	50	23
4	0.0027	Melted	50	33
5	0.0005	Solid	100	23
6	0.0015	Solid	100	33
7	0.0013	Melted	100	23
8	0.0006	Melted	100	33
9	0.0003	Solid	150	23
10	0.0006	Solid	150	33
11	0.0023	Melted	150	23
12	0.0020	Melted	150	33

\* In 4-5 cc. portions.

solvent to remove all the fat is shown in the following experiment, the results of which are given in Table 2. Samples of butterfat were warmed for a few minutes in platinum dishes, then washed through Gooch crucibles containing different quantities of asbestos with 100 cc. of petroleum ether in 10 cc. portions.

TABLE 2.  
*Butterfat retained by asbestos crucible.*

SAMPLE NO.	WEIGHT OF SAMPLE	WEIGHT OF ASBESTOS	FAT RETAINED BY ASBESTOS		
			<i>grams</i>	<i>per cent</i>	<i>grams per one gram of asbestos</i>
1	3.8	0.16	0.0013	0.03	0.0081
2	4.1	4.1	0.0272	0.66	0.0066
3	2.1	0.18	0.0007	0.03	0.0038
4	2.1	3.7	0.0212	1.01	0.0058
5	1.2	0.22	0.0002	0.02	0.0009
6	1.2	3.1	0.0198	1.64	0.0064
7	2.0	2.2	0.0086	0.45	0.0040
8	1.9	0.5	0.0028	0.15	0.0056
9	2.1	1.8	0.0081	0.38	0.0046
10	2.2	0.6	0.0030	0.13	0.0052
11	3.1	1.4	0.0091	0.30	0.0065
12	3.2	1.1	0.0062	0.20	0.0056

(c) *Volatilization of sodium chloride during ashing.*—Although the determination of non-fat solids is usually sufficient without a determination of sodium chloride, it is sometimes desirable to make the latter. In running a number of samples in an electric muffle, it is difficult to observe the precautions given in the official method for ashing. Loss of sodium chloride during ashing was indicated when duplicate samples, which

checked well for moisture and non-fat solids, showed discrepancies in values for ash.

(d) *Corrosion of the dish by butter during drying.*—This condition is observed when butter is dried in metal dishes other than platinum, particularly lead or aluminum dishes. Lead or aluminum dishes so used and then cleaned and dried have been found to appear discolored and corroded and to lose weight on continued use. The results given in Table 3 are illustrative.

TABLE 3.  
*Loss in weight of lead dishes during one analysis.*

LEAD DISH	LOSS IN WEIGHT	LOSS CALCULATED TO AVERAGE WEIGHT OF SAMPLE USED—2 GRAMS
No.	grams	per cent
1	0.0047	0.24
2	0.0042	0.21
3	0.0041	0.21
4	0.0030	0.15
5	0.0029	0.15
6	0.0031	0.16
7	0.0085	0.43
8	0.0053	0.27
9	0.0068	0.34
10	0.0073	0.37
11	0.0084	0.42
12	0.0061	0.31

(e) *Solution of salt.*—During the transfer and washing, the dish and crucible become so cold, owing to the rapid evaporation of the solvent, that moisture condenses on the apparatus and may dissolve some of the salt when the latter is present.

(f) *Drying to constant weight.*—The official method for moisture<sup>1</sup> states in part, “ \* \* \* dry at the temperature of boiling water, and weigh at hourly intervals until the weight becomes constant”. In addition to the inconvenience and loss of time involved in removing the dishes at hourly intervals, cooling, and weighing, the directions call for drying to constant weight. It has been the experience of the writers in analyzing several thousand samples of butter, that in general butter loses weight on drying for 1–4 hours, depending on the nature of the butter, the method of preparation of the sample for analysis, the size of the sample dried, the kind of drying dish, and the number of samples dried in the oven at one time. (“Synthetic” butter, prepared from dried butterfat, powdered skimmed milk, salt, and water, may lose weight for 24 hours or longer.) When the minimum weight has been reached, the butter gains weight at the rate of 0.3–0.6 mg. per hour (for samples weighing approximately 2 grams).

A series of six samples of butter weighing about 2.0 grams each was

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 276.

dried and weighed at intervals of 3 hours, 21 hours, and 120 hours. The average gain in weight from the 3rd to the 21st hour was 6.0 mg., or 0.30 per cent. From the 21st until the 120th hour the samples lost weight, the loss ranging from 1.20–2.54 per cent and averaging 2.10 per cent.

Samples of butter prepared by the stirrer method<sup>1</sup> and dried in flat metallic dishes will reach minimum weight in 1.5–2.5 hours. It has been found, in testing a large number of samples, that drying a sample so prepared for 2 hours and accepting the result as the minimum weight will give as close to the true value as will be obtained by drying at hourly intervals to minimum weight.

In addition to the above-mentioned possible sources of error, the official methods have certain drawbacks. Such are the time consumed, the inconvenience of transferring the sample during analysis, the scarcity of platinum—the best drying dish—and the undesirability of using the solvent petroleum ether because of its flammability and comparatively high cost. It has been attempted, therefore, (1) to develop a quick method for the direct determination of the most important constituent, the fat; (2) to find a suitable drying dish to replace the platinum dish; (3) to find a more satisfactory solvent than petroleum ether; and (4) to eliminate the transfer of the sample during analysis.

#### DIRECT DETERMINATIONS OF FAT.

The official method for the direct determination of fat requires preliminary drying, and the subsequent extraction with absolute ether or petroleum ether involves several difficulties. Unless suction is used filtration is slow, and it is difficult to wash all the fat out of the asbestos pad. If suction is applied, an unwieldy bell-jar must be used. The fat tends to creep, and it is very difficult to wash all of it down into the receiving flask.

Attempts to develop a satisfactory quick method for the direct determination of fat were unsuccessful.

#### DRYING DISHES.

As a drying dish, platinum is superior to any other type of dish, except in one respect—the cost. There are few laboratories that have as many as a dozen platinum dishes. The bad features of lead dishes have been discussed. Aluminum dishes have the necessary rigidity and heat conductivity, but they are sometimes attacked by some of the constituents in butter. It is difficult to remove all the salt from the dish if it becomes corroded.

Flat-bottomed silica dishes were found satisfactory so far as corrosion and ease of handling are concerned. The non-fat solids were removed

<sup>1</sup> *This Journal*, 1925, 8: 574.

quite easily from the dish by the solvent. The time required for drying the butter, however, was somewhat longer than was necessary for metal dishes. In view of the hourly weighings required by the official method this is a serious drawback.

Porcelain evaporating dishes are not corroded, nor do they retain any of the non-fat solids, but they are likely to chip and they require about twice as long a drying time as metal dishes.

#### THE SOLVENT.

Petroleum ether is undesirable as a solvent because it is very volatile, causing an inefficient recovery of solvent and preventing its use at a temperature above the melting point of butter; it has a great tendency to creep; it is flammable; it is not a definite chemical compound; it evaporates so rapidly that moisture condenses on the cooled surface of the dish or crucible in which it is used, making possible a loss of salt due to solution in water; and unless the asbestos pad is very thin (0.2 gram), the petroleum ether fails to remove all the fat from the pad.

Absolute ether has most of the drawbacks of petroleum ether, and it is more expensive.

After the properties of various fat solvents were investigated, a study was made of the suitability of carbon tetrachloride. This solvent was found to be superior to petroleum ether in the following respects: it is non-flammable; it is a definite chemical compound; it boils about 25°C. below the ordinary drying temperature and is therefore easily evaporated from the asbestos pad; it boils at a temperature sufficiently above the melting point of butter so that it may be used warm (60°-70°C.); it may be recovered almost quantitatively and in consequence is considerably cheaper than petroleum ether. The chief disadvantage involved in its use is its toxicity, which will be discussed later.

The relative solvent properties of petroleum ether and carbon tetrachloride are shown in Table 4 to be of the same order of efficiency.

TABLE 4.  
*Solvent efficiency of carbon tetrachloride and petroleum ether.*

SAMPLE NO.	SOLVENT	WEIGHT OF ASBESTOS	WEIGHT OF BUTTERFAT TAKEN	WEIGHT OF BUTTERFAT RETAINED	AMOUNT OF SOLVENT (IN 5 CC. PORTIONS)
		grams	grams	grams	cc.
1	Petroleum ether	0.5	2.	0.0022	100
2	Petroleum ether	0.5	2.	0.0118	100
3	Petroleum ether	0.4	1.5	0.0022	75
4	Petroleum ether	0.4	1.	0.0025	75
5	Petroleum ether	1.0	2.	0.0056	200
6	Petroleum ether	2.0	2.5	0.0080	200
7	Carbon tetrachloride	0.5	2.	0.0018	100
8	Carbon tetrachloride	0.5	2.	0.0016	100
9	Carbon tetrachloride	0.4	1.5	0.0030	75
10	Carbon tetrachloride	0.4	1.	0.0038	50
11	Carbon tetrachloride	1.0	2.	0.0046	100
12	Carbon tetrachloride	2.0	2.5	0.0075	100

## ELIMINATION OF TRANSFER AFTER DRYING.

The so-called "Gooch method" has often been used instead of the official method; it differs from the official method in that the butter is weighed in a Gooch crucible about three-fourths full of dry ignited asbestos. In some cases a cover glass is placed under the crucible while it is in the drying oven to catch any fat that might not be absorbed. The fat is then extracted with petroleum ether. The Gooch method is shorter than the official in that one weighing is eliminated, and it is not necessary to transfer the solids from the drying dish to a Gooch crucible.

It has been known in this laboratory for several years<sup>1</sup> that the Gooch method is inaccurate because of the relatively large amount of fat retained in the asbestos. Recently the method was tested<sup>2</sup> in another laboratory, and the conclusion was reached that the method was unreliable, owing to the impossibility of washing all the fat out of the asbestos.

TABLE 5.

*Fat retained in the asbestos-Gooch method.*

A

(Butterfat washed with 50 cc. of kerosene at 100°C., followed with 25 cc. of petroleum ether.)

SAMPLE NO	WEIGHT OF SAMPLE	WEIGHT OF ASBESTOS	FAT RETAINED BY ASBESTOS	
	grams	grams	grams	per cent
1	1.5	2.0	0.0027	0.18
2	1.6	2.0	0.0097	0.56
3	1.4	2.0	0.0025	0.18
4	2.0	3.0	0.0171	0.84
5	1.0	3.0	0.0035	0.19
6	2.1	3.0	0.0084	0.41
7	3.0	3.0	0.0130	0.43
8	4.0	4.0	0.0129	0.32
9	5.1	4.0	0.0164	0.53
10	6.0	4.0	0.0131	0.24

(Butterfat washed with 100 cc. carbon tetrachloride at 60°-70°C., through 2.0 gram asbestos. Sample. 2.0-3.2 grams.)

1.....	0.0037	0.12
2.....	0.0035	0.11
3.....	0.0015	0.07
4.....	0.0017	0.08
5.....	0.0050	0.22
6.....	0.0022	0.08
7.....	0.0017	0.07
8.....	0.0027	0.10
9.....	0.0042	0.20
10.....	0.0034	0.15
11.....	0.0043	0.14
12.....	0.0019	0.08

<sup>1</sup> D. B. Blabec. Unpublished report, 1923.

<sup>2</sup> T. W. Ferris. Unpublished report, May, 1925.

The possibilities of this method were examined, and several modifications were tried, such as the use of hot kerosene (100°C.) followed by petroleum ether, and of hot carbon tetrachloride (50°–70°C.). The results in Table 5 show that neither of the treatments described removed all the fat from the asbestos.

Since the desideratum is a filter material that will retain the fat during drying but will allow it to be readily and completely extracted, an attempt was made to find such a material. Fullers' earth, glass wool-asbestos combinations, alundum crucibles, filter paper pulp, and sea-sand were tried without success.

The most promising material was a good grade of white sand obtained in Minneapolis<sup>1</sup>. To retain the butterfat during drying, it was found that the sand must be of sufficient fineness to pass through a 100-mesh sieve. The sand was prepared by extracting it, in the order named, with water, strong hydrochloric acid, water, 20 per cent sodium hydroxide solution, water, and strong hydrochloric acid, and finally washing thoroughly with water and igniting. This procedure removed interfering substances, particularly iron compounds.

The Gooch crucibles were prepared by putting 15–18 grams of the prepared sand onto a 0.1 gram pad of asbestos. In the analysis, a 1.0–1.5 gram sample of butter was weighed into a crucible so prepared, dried 2 hours at the temperature of boiling water, cooled, and weighed. The Gooch crucible was then warmed in an oven, and the dried fat was extracted with 100 cc. of carbon tetrachloride at 60°–70°C. in 5–10 cc. portions, slight suction being used. Care was taken to keep the sand moist with the solvent while suction was applied, since some sand may be lost as a fine spray if suction is applied to a dry sand-Gooch crucible. The crucible was dried for 30 minutes, cooled, and weighed. When desired, salt was determined by washing it out of the crucible with water and titrating against a standard silver nitrate solution, with potassium chromate as indicator. The crucible was prepared for further use by igniting in a muffle, washing with water, and drying.

During the past year many samples have been analyzed by this method (see Table 6), check analyses being made by the official method under the best conditions.

The best conditions for the official method were found to be:

*Moisture.*—Dry 1.0–1.5 grams of the sample prepared by the stirrer method in a flat-bottomed platinum dish having a diameter of at least 5 cm. for 2 hours at the temperature of boiling water, cool, and weigh. Calculate the loss in weight as moisture.

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<sup>1</sup> Through the courtesy of William Rabak.

TABLE 6.  
Comparison of sand-Gooch method with official method.

SAMPLE NO.	MOISTURE			NON-FAT SOLIDS			FAT (BY DIFFERENCE)		
	Official	Sand-Gooch	Difference*	Official	Sand-Gooch	Difference	Official	Sand-Gooch	Difference
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	14.96	15.05	-0.09	3.07	3.09	-0.02	81.97	81.86	+0.11
2	14.77	14.61	+0.16	3.64	3.67	-0.03	81.59	81.72	-0.13
3	15.07	14.92	+0.15	3.77	3.67	+0.10	81.16	81.41	-0.25
4	15.21	15.13	+0.08	4.47	4.51	-0.04	80.32	80.36	-0.04
5	15.27	15.18	+0.09						
6	15.77	15.70	+0.07	3.90	3.69	+0.21	80.33	80.61	-0.28
7	14.78	14.80	-0.02	3.47	3.56	-0.09	81.75	81.64	+0.11
8	14.71	14.92	-0.21	3.42	3.54	-0.12	81.87	81.54	+0.33
9	15.09	15.08	+0.01	3.06	3.22	-0.16	81.85	81.70	+0.15
10	15.17	15.11	+0.06	3.80	3.85	-0.05	81.03	81.04	-0.01
11	16.16	16.08	+0.08	3.72	3.81	-0.09	80.12	80.11	+0.01
12†	15.70	15.76	-0.06	3.78	3.88	-0.10	80.52	80.36	+0.16
13	15.46	15.42	+0.04	3.66	3.63	+0.03	80.88	80.95	-0.07
14	15.39	15.33	+0.06	3.48	3.48	±0.00	81.13	81.19	-0.06
15	16.16	16.17	-0.01	3.40	3.40	±0.00	80.44	80.43	+0.01
16	14.16	14.16	±0.00	4.17	4.18	-0.01	81.67	81.66	+0.01
17	14.36	14.31	+0.05	4.38	4.40	-0.02	81.26	81.29	-0.03
18	15.38	15.29	+0.09	3.41	3.44	-0.03	81.21	81.27	-0.06
19	17.56	17.52	+0.04	3.19	3.21	-0.02	79.25	79.27	-0.02
20	14.76	14.79	-0.03	3.41	3.46	-0.05	81.83	81.75	+0.08
21	15.37	15.33	+0.04	3.19	3.22	-0.03	81.44	81.45	-0.01
22	15.71	15.72	-0.01	3.06	3.12	-0.06	81.23	81.16	+0.07
23	15.41	15.43	-0.02	3.07	3.22	-0.15	81.52	81.35	+0.17
24	15.66	15.65	+0.01	3.12	3.27	-0.15	81.22	81.08	+0.14
25	15.21	15.21	±0.00	2.66	2.60	+0.06	82.13	82.19	-0.06
26	14.97	14.94	+0.03	2.56	2.56	±0.00	82.47	82.50	-0.03
27	15.39	15.34	+0.05	2.77	2.67	+0.10	81.84	81.99	-0.15
28	15.46	15.43	+0.03	2.73	2.66	+0.07	81.81	81.91	-0.10
29	15.68	15.71	-0.03	2.63	2.65	-0.02	81.69	81.64	+0.05
30	14.19	14.16	+0.03	3.88	3.83	+0.05	81.93	82.01	-0.08
31	14.07	14.05	+0.02	3.81	3.76	+0.05	82.12	82.19	-0.07
32	14.55	14.53	+0.02	3.82	3.88	-0.06	81.63	81.59	+0.04
33	14.29	14.25	+0.04	3.81	3.89	-0.08	81.90	81.86	+0.04
34	14.11	14.10	+0.01	3.79	3.80	-0.01	82.10	82.10	±0.00
35	15.97	15.85	+0.12	2.80	2.88	-0.08	81.23	81.27	-0.04
36	15.46	15.40	+0.06	2.41	2.50	-0.09	82.13	82.10	+0.03
37	14.94	14.92	+0.02	3.65	3.64	+0.01	81.41	81.44	-0.03
38	14.61	14.57	+0.04	3.62	3.66	-0.04	81.77	81.77	±0.00
39	14.55	14.61	-0.06	3.58	3.64	-0.06	81.87	81.75	+0.12
40	13.19	13.25	-0.06	4.06	4.11	-0.05	82.75	82.66	+0.11
41	13.00	12.93	+0.07	3.71	3.64	+0.07	83.29	83.43	-0.14
42	14.19	14.09	+0.10	4.82	4.91	-0.09	80.99	81.00	-0.01
43	14.54	14.50	+0.04	3.78	3.63	+0.15	81.68	81.87	-0.19
44	16.52	16.50	+0.02	1.29	1.28	+0.01	82.19	82.22	-0.03
45	15.87	15.86	+0.01	1.25	1.22	+0.03	82.88	82.92	-0.04
46	17.30	17.43	-0.13	1.53	1.41	+0.12	81.17	81.16	+0.01
47	15.17	15.08	+0.09	0.89	0.92	-0.03	83.94	84.00	-0.06
48	16.08	15.95	+0.13	0.86	0.98	-0.12	83.06	83.07	-0.01
49	15.79	15.72	+0.07	0.76	0.91	-0.15	83.45	83.37	+0.08
50	16.24	16.16	+0.08	1.26	1.40	-0.14	82.50	82.44	+0.06
51	17.56	17.47	+0.09	0.99	1.10	-0.11	81.45	81.43	+0.02
52	16.25	16.28	-0.03	0.88	1.00	-0.12	82.87	82.72	+0.15

\* + Official &gt; Sand-Gooch.

- Official &lt; Sand-Gooch.

† Samples 1-12, 13-24, 25-34, 35-39, 40-52, 53-63, 64-66 analyzed at different times.



TABLE 6—Continued.

SAMPLE NO.	MOISTURE			NON-FAT SOLIDS			FAT (BY DIFFERENCE)		
	Official	Sand-Gooch	Difference*	Official	Sand-Gooch	Difference	Official	Sand-Gooch	Difference
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
53	16.25	16.15	+0.10	3.91	3.95	-0.04	79.96	79.90	+0.06
54	15.95	15.90	+0.05	3.80	3.75	+0.05	80.45	80.35	+0.10
55	16.03	16.10	-0.07	3.63	3.64	-0.01	80.18	80.26	-0.08
56	13.56	13.49	+0.07	3.58	3.60	-0.02	82.96	82.91	+0.05
57	13.39	13.34	+0.05	3.44	3.42	+0.02	83.31	83.24	+0.07
58	13.66	13.62	+0.04	3.41	3.42	-0.01	82.99	82.96	+0.03
59	13.76	13.82	-0.06	2.12	2.02	+0.10	84.20	84.16	+0.04
60	13.86	13.86	0.00	2.11	2.04	+0.07	84.17	84.10	+0.07
61	15.10	15.20	-0.10	2.92	2.80	+0.12	82.02	82.00	+0.02
62	14.65	14.75	-0.10	3.01	3.09	-0.08	81.98	82.16	-0.18
63	14.71	14.80	-0.09	3.19	3.21	-0.02	81.88	81.99	-0.11
64	15.13	15.14	-0.01	3.89	3.68	+0.21	81.38	81.18	+0.20
65	15.12	15.17	-0.05	3.41	3.33	+0.08	81.53	81.50	+0.03
66	15.09	15.14	-0.05	3.43	3.44	-0.01	81.36	81.42	-0.06

Maximum difference ..... +0.33 to -0.28

Average difference (irrespective of sign) ..... 0.08

\* + Official > Sand-Gooch.

- Official < Sand-Gooch.

*Non-fat solids.*—Dissolve the dry, melted butterfat obtained from the moisture determination in petroleum ether, transfer to a weighed Gooch crucible containing 0.10–0.15 gram of asbestos with petroleum ether from a wash bottle, and wash until free from fat, being careful to wash off any fat that has crept up on the crucible. Dry the crucible and contents 30 minutes at the temperature of boiling water, cool, and weigh.

*Fat.*—Calculate the fat by difference.

#### TOXICITY OF CARBON TETRACHLORIDE.

The medical literature on the toxicity of carbon tetrachloride is meager<sup>1</sup>. It is reported by some authorities as being twice as toxic as chloroform, and by others as being half as toxic. Its use to induce narcosis ("carbena jag") has been described in the literature. The vapors are undoubtedly irritant to the respiratory tract, particularly in an enclosed space. The only bad effect noticed by the writers in a laboratory with three analysts, where carbon tetrachloride has been used for about two years, was a slight nervous tension after long continued use, accompanied in the case of one analyst by a headache. A desk hood or a fan was found to be of value in removing the vapor. In order to eliminate any possible danger, several closed-system ex-

<sup>1</sup> A. D. H., *Brit. Med. J.*, 1920, 11: 497; Colman and Marshall, *Lancet*, 1907, 1: 1709; Foster, *Public Health Rept.*, 1918, 33: 1823; Hall, *J. Agr. Research*, 1921, 21: 157; Lamson, *J. Pharmacol.*, 1923, 22: 215; Lehmann, *Arch. Hyg.*, 1911, 74: 1; Peterson, Haines and Webster, *Legal Medicine and Toxicology*, 1925, 2: 794; Starr, *J. Ind. Hyg.*, 1922, 4: 203; Veley, *Lancet*, 1909, 2: 1162; Waller, *Ibid.*, 369, 1307; *J. Am. Med. Assoc.*, 1924, 83: 461, 705, 1022.

tractors were devised and tried out. The one illustrated in Figure 1 proved to be the simplest, safest, and most convenient. There is practically no loss of solvent.

#### SAND-GOOCH CONTINUOUS EXTRACTION METHOD

The details of the method are as follows:

Weigh accurately 1.0–1.5 grams of the prepared sample in a sand-Gooch, dry for two hours at the temperature of boiling water, cool, weigh, and extract in the apparatus illustrated in Fig. 1 for 30–40 minutes, adjusting the heat so that the solvent drops into the crucible at the same rate as the crucible drains. Draw out most of the solvent remaining in the crucible by applying suction for a few seconds, then dry the crucible for 30 minutes at 100°C.

Three lots of butter were prepared by the stirrer method, and twelve samples were taken from each jar, six being run by the official method and six by the sand-Gooch method, as described previously. The results, given in Table 7, show that the proposed method yields values identical with those obtained by the official method.

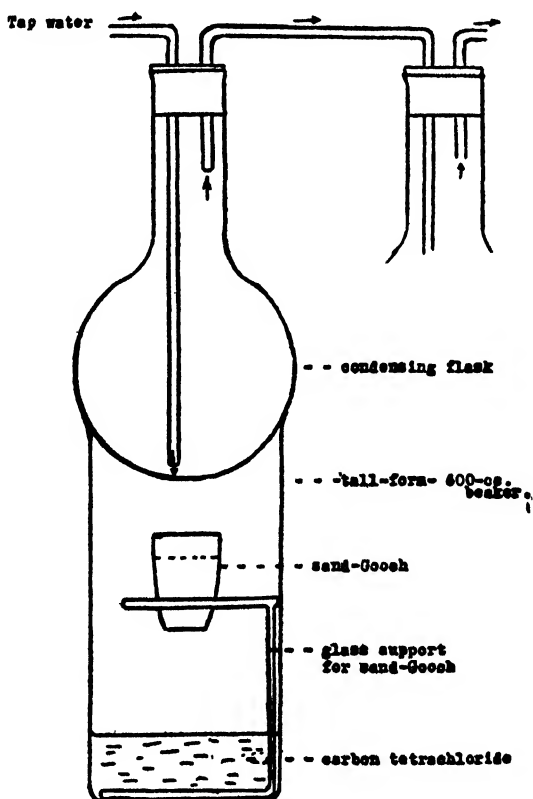


FIG. 1. DESIGN OF CLOSED-SYSTEM EXTRACTOR THAT PROVED MOST SATISFACTORY.

TABLE 7.

*Comparison of sand-Gooch continuous extraction method with official method.*

SAMPLE NO.	JAR NUMBER	MOISTURE			NON-FAT SOLIDS			FAT <sub>2</sub> (BY DIFFERENCE)		
		Sand-Gooch	Official	Difference*	Sand-Gooch	Official	Difference	Sand-Gooch	Official	Difference
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	I	14.15	14.20	+0.05	3.79	3.81	+0.02	82.06	81.99	-0.07
2		14.16	14.25	+0.09	3.73	3.72	-0.01	82.11	82.03	-0.08
3		14.16	14.25	+0.09	3.76	3.73	-0.03	82.08	82.02	-0.06
4		14.18	14.14	-0.04	3.80	3.72	-0.08	82.02	82.14	+0.12
5		14.16	14.22	+0.06	3.72	3.74	+0.02	82.12	82.04	-0.08
6		14.12	14.18	+0.06	3.79	3.86	+0.07	82.09	81.96	-0.13
Average		14.16	14.21	+0.05	3.77	3.76	-0.01	82.07	82.03	-0.04
1	II	14.12	14.16	+0.04	4.00	3.97	-0.03	81.88	81.87	-0.01
2		14.10	14.13	+0.03	4.04	4.03	-0.01	81.86	81.84	-0.02
3		14.17	14.17	±0.00	4.02	4.03	+0.01	81.81	81.80	-0.01
4		14.14	14.20	+0.06	4.02	4.00	-0.02	81.84	81.80	-0.04
5		14.14	14.15	+0.01	4.05	4.02	-0.03	81.81	81.83	+0.02
6		14.13	14.16	+0.03	4.06	4.02	-0.04	81.81	81.82	+0.01
Average		14.13	14.16	+0.03	4.03	4.01	-0.02	81.84	81.83	-0.01
1	III	13.95	14.02	+0.07	3.86	3.85	-0.01	82.19	82.13	-0.06
2		13.86	13.92	+0.06	3.89	3.83	-0.06	82.25	82.25	±0.00
3		13.89	13.95	+0.06	3.92	3.90	-0.02	82.21	82.15	-0.06
4		13.92	13.90	-0.02	3.89	3.89	±0.00	82.19	82.21	+0.02
5		13.91	13.92	+0.01	3.85	3.86	+0.01	82.24	82.22	-0.02
6		13.84	13.88	+0.04	3.90	3.93	+0.03	82.26	82.25	-0.01
Average		13.90	13.93	+0.03	3.88	3.88	±0.00	82.22	82.19	-0.03
Grand average		14.06	14.10	+0.04	3.89	3.88	-0.01	82.04	82.02	-0.02

Maximum difference ..... +0.12 to -0.13  
 Average difference (irrespective of sign) ..... 0.04

\* + Official > Sand-Gooch.  
 - Official < Sand-Gooch.

## SUMMARY AND CONCLUSIONS.

1. A critical study of the official methods for the analysis of butter is presented, and some possible sources of error are pointed out.

2. The sand-Gooch continuous extraction method described is proposed as an alternative method for the analysis of butter.

3. When a large number of samples are being run, the proposed method has been found by direct comparison to require considerably less time than the official method.

4. The sand-Gooch continuous extraction method and the official method yield results that are in close agreement.

## THE SEPARATION OF FORMIC ACID IN FOOD PRODUCTS BY DISTILLATION WITH XYLENE.

By JAMES K. MORTON and G. C. SPENCER (Analytical Reagent Investigations Laboratory, Bureau of Chemistry, Washington, D. C.).

In the examination of foodstuffs for formic acid one of the difficulties encountered is its separation from the materials that contain it. The usual method of effecting this separation is that of Fincke<sup>1</sup> by steam distillation.

In the determination of acetic acid in pyroligneous acid, Grotlisch<sup>2</sup> effected the separation of the volatile constituents by distilling the mixture with an excess of xylene. This carried over into the receiving flask acetic and formic acids and other volatile material. The formic acid in this distillate may be determined quantitatively without the removal of the xylene or acetic acid by reduction of mercuric chloride to mercurous chloride; the mercurous chloride is then separated and weighed, or titrated in a strong hydrochloric acid solution with a standard solution of potassium iodate, as suggested by Jamieson<sup>3</sup>. In the absence of other reducing substances the quantity of mercurous chloride obtained is an accurate measure of the quantity of formic acid in the solution.

A report of the probable amounts of reducing substances other than formic acid, present in certain food products, was made by Seeker<sup>4</sup> as referee on this subject for the Association of Official Agricultural Chemists.

Mixed organic acids, natural fruit juices, and certain food products that do not contain acetic fermentation products lend themselves readily to this process of separation. The process of acetic fermentation as illustrated in the acetification of cider to vinegar produces a substance that has been identified by Balcom<sup>5</sup> as dimethyl ketol. This material is volatile and has high reducing properties, and its separation from formic acid is best effected by steam distillation.

In the distillation with xylene it is essential that all the water in the sample be removed and carried over with the xylene into the receiving flask. Large volumes of the sample may be taken for analysis, but the solution in the distilling flask must be concentrated to a small volume before the xylene is added. Small adhering drops of moisture that condense on the upper inner surface of the neck of the distilling flask and connecting tube retain an appreciable quantity of formic acid. These drops are gradually absorbed and carried over with the xylene vapor. The distillation should be conducted entirely under reduced pressure at

<sup>1</sup> *Z. Nahr. Genussm.*, 1911, 21: 1; 22: 88; 1912, 23: 255; 1913, 25: 386.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1920, 12: 1183.

<sup>3</sup> *Am. J. Sci.*, 1912, 33: 349.

<sup>4</sup> *This Journal*, 1915, 1: 210, 556.

<sup>5</sup> *J. Am. Chem. Soc.*, 1917, 39: 309.

as low a temperature as possible, preferably in an oil bath, to prevent local overheating and consequent charring. The distillate is collected in a barium carbonate suspension.

#### APPARATUS.

*Digestion flask.*—500 cc. Pyrex Kjeldahl.

*Condenser.*—15 inch spiral with connecting tubes.

*Aspirating flask.*—Ordinary 500 cc. filter flask.

#### REAGENTS.

(a) *Chloroform.*

(b) *Hydrochloric acid.*—36 per cent.

(c) *Barium carbonate suspension.*—2 grams of  $\text{BaCO}_3$  to 50 cc. of water.

(d) *Mercuric chloride solution.*— $\text{HgCl}_2$ , 100 grams and  $\text{NaCl}$ , 30 grams to 1 liter of water.

(e) *Potassium iodate solution.*—2.326 grams of  $\text{KIO}_3$  to 1 liter of water (check with 0.1 N sodium thiosulfate).

(f) *Sodium acetate solution.*—30 per cent.

#### PROCEDURE.

Connect the 500 cc. distilling flask to a vertical spiral condenser. Provide an air intake closed by a pinchcock in the stopper of the distilling flask to be used later in equalizing pressure after the removal of the flame. Let the outlet tube of the condenser, extended if necessary, pass through a stopper into the neck of the aspirating flask sufficiently to dip under the surface of the barium carbonate suspension in the flask. Make all connections with a view to subsequent distillation at reduced pressure.

For liquids take 100 cc. of the sample; for semi-solids take 50 grams of the sample and add 50 cc. of water.

Place the measured sample in the distilling flask and add a few pieces of unglazed porcelain and about 0.5 gram of tartaric acid. Place 50 cc. of the barium carbonate suspension in the aspirating flask and connect the entire apparatus as directed. First concentrate the solution in the distilling flask as much as possible without charring; this operation at the same time distills over some of the formic acid. Interrupt the distillation and when sufficiently cool add to the distilling flask 100 cc. of xylene and continue as before. Repeat with 100 cc. more of xylene, which removes the last of the formic acid. Without separating the xylene, filter out the excess of barium carbonate by suction and wash with 20–30 cc. of water. Transfer the filtrate to a 500 cc. Erlenmeyer flask. Make acid with acetic acid and add 10 cc. of the sodium acetate solution and 10 cc. of the mercuric chloride solution. Stopper the flask with a cork, through which is inserted 2 feet of one-quarter inch glass tubing as an air condenser. Place the flask inside the steam bath and heat at steam temperature for two hours. Filter hot with suction, collect the precipitate on an asbestos mat in a Gooch crucible, and wash the flask and precipitate thoroughly with hot water. (The color of the precipitated mercurous chloride should be white; if dark or yellow it indicates contamination or reducing substances other than formic acid.) Transfer the contents of the Gooch crucible, asbestos mat and all, back into the same flask, using not more than 10–15 cc. of water. Add 30–40 cc. of strong hydrochloric acid and 10 cc. of chloroform and titrate with the standard solution of potassium iodate. The end point is indicated by the discharge of the violet coloration in the chloroform, which does not return after one minute of brisk agitation.

*Analytical data.*

MATERIAL	QUANTITY TAKEN	FORMIC ACID ADDED	FORMIC ACID FOUND	FORMIC ACID CALCULATED IN SAMPLE*
		<i>grams</i>	<i>grams</i>	<i>per cent</i>
Acetic acid, Tech.....	10 cc.	none	0.0351	0.351
	20 cc.	none	0.0706	0.353
	10 cc.	0.01104	0.0450	0.340
	10 cc.	0.00736	0.0422	0.349
Ketchup.....	100 gms.	none	0.0048	0.0048
	50 gms.	0.00638	0.0100	0.007
	50 gms.	0.0319	0.0341	0.007
Apple stock.....	50 gms.	none	none	none
	50 gms.	0.00638	0.0063	nil
	50 gms.	0.0319	0.0317	nil
Grape juice.....	25 cc.	0.0319	0.0316	....
	25 cc.	0.0319	0.0308	....
Elderberry juice.....	100 cc.	none	0.0014	0.0014
	50 cc.	0.056	0.0586	0.0052
	50 cc.	0.028	0.029	0.002
Elderberry juice (Fincke method)....	50 cc.	0.028	0.0287	0.0014
Grape fruit juice.....	100 cc.	none	0.0034	0.0034
Pineapple juice.....	50 cc.	none	0.0033	0.0066

\* Specific gravity of sample disregarded. Cubic centimeters taken as grams in calculating percentage. Correction made for added formic acid before calculating percentage.

## SUMMARY.

The xylene distillation method for separating acetic acid from mixtures is adapted to the separation of formic acid from food products. In the absence of other volatile reducing substances the results obtained compare favorably with the results obtainable by the steam distillation method. The manipulation is simpler and less time is required.

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in vinegar:

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## DETERMINATION OF STRYCHNINE IN POISONED GRAINS.

By JOHN W. ELMORE (Division of Chemistry, California State Department of Agriculture, Sacramento, Calif.).

In connection with the enforcement of the California Economic Poison Law it became necessary to determine the percentage of strychnine in the poison baits used in rodent control. Such materials as raisins, wheat, and particularly barley, are coated or impregnated with the alkaloid and employed in considerable quantity against gophers and California ground squirrels. The manufacturers of these materials are obliged to state on the containers the percentage of the active ingredients, or the percentage of the inert ingredients, or both. The percentage of strychnine is usually stated.

A method of analysis that was used in control work by one of the firms manufacturing poisoned barley was temporarily adopted by this laboratory at the time the law went into effect. It was based on the solubility of strychnine in hot kerosene and was carried out as follows:

Introduce 25 grams of the poisoned grain into a 250 cc. Erlenmeyer flask. Moisten with chloroform and add enough kerosene to more than cover the grain. Hold the flask in a clamp and heat over an open flame, shaking continuously. While still hot, filter into a dry Erlenmeyer flask. Repeat twice more but with the omission of the chloroform. When cool enough, add 100 cc. water and cochineal indicator. Close the flask with a stopper and shake vigorously. Titrate with 0.1 *N* sulfuric acid, adding small portions at a time and shaking vigorously after each addition. Each cc. of 0.1 *N* sulfuric acid is equivalent to 0.0334 gram of strychnine.

This method was checked against another method which had been used in this laboratory based upon the extraction of the alkaloid with chloroform as follows:

Extract 10 grams of the material with chloroform in a Soxhlet apparatus for 4 hours. Evaporate to dryness over steam. Extract with 10 cc. of water and a few drops of acetic acid, boil, cool, and filter. Repeat the extraction and wash. Concentrate the

filtrate to 10 cc., cool, and add 10 cc. of cold saturated picric acid solution. Filter the precipitate on a Gooch crucible, wash with the smallest possible quantity of water, and dry at 105°C. Weight of strychnine picrate  $\times 0.5932$  = strychnine.

The two methods were compared on laboratory sample No. 4048, having a guarantee of 0.32 per cent strychnine, with the following results:

	<i>per cent</i>
Kerosene method.....	0.254
	0.261
Soxhlet method.....	0.254

The results by the two methods evidently agreed, and as the kerosene method was the shorter, a large number of analyses were carried through by it. The results, however, appeared to be consistently low, judging from the guaranteed percentage in the materials examined. The following are typical:

LABORATORY NO.	GUARANTEED <i>per cent</i>	FOUND <i>per cent</i>
4089	0.20	0.21
4090	0.20	0.21
4098	0.3125	0.25
4099	0.30	0.23
4100	0.25	0.24
4107	0.32	0.28
4109	0.31	0.21
4113	0.25	0.19

In view of these figures and others similar to them, a closer examination of the methods used was decided upon. A small quantity of poisoned barley was therefore made up according to the following formula issued by the Bureau of Biological Survey, U. S. Department of Agriculture<sup>1</sup>:

Barley . . . . .	16 quarts
Strychnine. . . . .	1 ounce
Bicarbonate of soda. . . . .	1 ounce
Saccharin... . . . .	1/10 ounce
Heavy corn sirup.... .	1/4 pint
Thin starch paste.... .	3/4 pint
Glycerine.....	1 tablespoonful

Allowing for the strychnine lost by sticking to the utensils, the theoretical quantity of alkaloid in the finished material was 0.253 per cent. Analysis gave the following results:

<sup>1</sup> Bi—141, Nov. '20.



	<i>per cent</i>
Kerosene method.....	0.187
	0.187
Soxhlet method.....	0.244
	0.225
	0.218

It was thought best, therefore, to abandon the kerosene extraction and search for the source of error in the Soxhlet method.

Very little information could be obtained about the precipitation and properties of strychnine picrate which was the final product weighed in this method. To test whether different conditions of acidity during the precipitation or different volumes might possibly influence the composition of the compound, 2 grams of pure strychnine alkaloid were made up to 200 cc. with a little acetic acid and several 20 cc. portions of this solution, representing 0.20 gram of strychnine, were taken for analysis. The following variations of conditions were noted:

Twenty cc. of solution without further dilution and containing excess acetic acid. Picric acid solution added rapidly.

Recovered 0.2003 gram of strychnine.

Twenty cc. of solution without further dilution and containing trace of acetic acid. Picric acid solution added rapidly.

Recovered 0.2009 gram of strychnine.

Twenty cc. of solution without further dilution and containing trace of acetic acid. Picric acid solution added slowly.

Recovered 0.1991 gram of strychnine.

Twenty cc. of solution diluted to 100 cc. and containing trace of acetic acid. Picric acid solution added slowly.

Recovered 0.1997 gram of strychnine.

Evidently these variations in the conditions of precipitation did not have much effect on the composition of the strychnine picrate.

#### SOLUBILITY OF STRYCHNINE PICRATE.

Some work on the solubility of strychnine picrate was done at this time. In order to ascertain how much the precipitates could be washed without danger of loss from solubility, two crucibles containing precipitates were washed with 50 cc. portions of cold water. The precipitates were obtained in the usual course of analysis and contained small amounts of impurities, as shown by their darker color. Pure strychnine picrate is a lemon yellow. The crucibles were dried and weighed between each leaching.

	WEIGHTS OF CRUCIBLES	LOSS
	grams	gram
A.....	9.4002	
	9.3998	0.0004
	9.3983	0.0015
	9.3978	0.0005
B.....	17.8328	
	17.8312	0.0016
	17.8305	0.0007
	17.8283	0.0012
	17.8272	0.0011

This gives a loss of a little over 1 mg. per 50 cc. of wash water. Similar results were obtained with a precipitate obtained from pure strychnine and picric acid. As 50–80 cc. is sufficient water to thoroughly wash a precipitate on a small Gooch crucible, evidently there was no danger of appreciable loss in this part of the analysis. It should be noted that since the solubility increased with the temperature, cold water should be used in the washing.

It seemed possible that some of the ingredients of the poison paste, which was applied to the barley, might be affecting the Soxhlet extraction. A quantity of the paste, therefore, was made up, but instead of applying it to barley it was diluted to 200 cc., and an amount corresponding to 0.2762 gram of strychnine was applied to some glass beads and dried. The beads were then extracted with chloroform in a Soxhlet apparatus, but only 0.175 gram and 0.202 gram, respectively, was recovered. They were subsequently washed with dilute hydrochloric acid, and the solution gave a positive test for strychnine with Mayer's reagent. Two-tenths gram of pure strychnine was then extracted from a Soxhlet thimble alone and 0.199 gram recovered. Another 0.2 gram portion was placed with some fresh dry barley in a Soxhlet thimble and extracted with chloroform, 0.197 gram being recovered. These results indicated that chloroform did not completely remove the strychnine from the dry barley and dry paste. The Soxhlet method was therefore abandoned.

Attempts were then made to extract the alkaloid from the poison grain by digesting it with dilute acetic acid, but the thick paste produced made further work impossible.

A search of the literature at this time disclosed the following method used in the assay of *nux vomica*<sup>1</sup>, which seemed to offer possibilities:

#### METHOD.

Take 12 grams of finely powdered *nux vomica*, place in a dry 200 cc. flask, add a mixture of 80 cc. of ether and 40 cc. of chloroform, and stopper tightly. After one-half hour, add 10 cc. of 10 per cent ammonium hydroxide and shake repeatedly for 1 hour. Fifteen to 20 cc. of water is then added in 2 or 3 portions with vigorous shaking, to agglutinate the drug and cause the ether-chloroform solution of the extracted alkaloids

<sup>1</sup> Allen. Commercial Organic Analysis, 1912, Vol. VI, p. 469.

to separate in a clear layer. Exactly 100 cc. of the latter clear liquid (representing 10 grams of drug) is then poured off and transferred to a separator. This is shaken out with 50 cc. of 0.5 per cent hydrochloric acid, and the extraction is repeated with 25 cc. portions of the latter until a few drops of the acid layer no longer give a cloudiness on addition of Mayer's reagent. The united acid layers are now filtered into a second separator, 30 cc. of a mixture of three parts of chloroform and one part of ether is added, the aqueous layer is rendered distinctly alkaline with an excess of ammonia, and the mixture is agitated thoroughly for 1 or 2 minutes. The separated chloroform layer is then run off, and the aqueous layer is washed with further portions of chloroform-ether.

The united extractions are evaporated and the alkaloid weighed.

It was thought a more direct method could be worked out depending on the weighing of the strychnine picrate; hence the method was applied as indicated up to the point where the hydrochloric acid extract was obtained. This was then evaporated and filtered, and the strychnine picrate was precipitated. After standing for some time, the latter was filtered off, washed, dried, and weighed.

This method applied to whole-coated grain gave fairly good results, those of the known material being 0.244 per cent and 0.251 per cent, as against 0.253 per cent present.

However, when this method was applied to whole grains in which the alkaloid had penetrated into the kernels, the results indicated that the extraction was not complete. The following are typical:

LABORATORY NO.	GUARANTEED per cent	FOUND per cent
4158 (apparently coated)	0.20	0.207
4159 (coated)	0.3125	0.320
4160	0.25	0.247
4197	0.30	0.278
4198	0.347	0.267
4200 (penetrated)	0.3	0.232
4201 (penetrated)	0.3	0.291

Several attempts were made to extract the alkaloid from the grain by hydrolyzing all the material with 10 per cent hydrochloric acid to remove the starch. The product was filtered, and a clear liquid was obtained, but upon attempting to extract the strychnine from the liquid with chloroform very troublesome emulsions were encountered. Examination of the solutions also indicated that some loss of strychnine had taken place during the prolonged treatment with hydrochloric acid.

Attempts were then made to apply Allen's method to the material after it had been ground finely. In doing this it was found that water could not be added to the mass as in the analysis of nux vomica powder, "to agglutinate the drug", since a thick paste was formed, but if a few cc. of a thick sirup, such as Karo, were added the solvent layer separated very well. The latter, however, was loaded with starch and gums. Upon extracting the strychnine from this solution with hydrochloric

acid, some of the impurities present also passed into the acid layer and made troublesome emulsions. Attempts were made to purify the alkalioid by extracting the hydrochloric acid solution with chloroform according to Allen's method for *nux vomica*, but the emulsions formed would not separate well even in a centrifuge. It was finally found possible to purify the hydrochloric acid solution with lead acetate and acetic acid.

In preparing the grain for analysis, it was usually necessary to dry it before grinding as it contained from 10–15 per cent of water. When the material was dried overnight at 100°C. it darkened somewhat, and the strychnine content was apparently reduced 0.03 or 0.04 per cent. It was thought best, therefore, to dry the samples at 60° or less.

In an effort to shorten the method, several attempts were made to eliminate the acid extraction by adding 100 cc. of 0.5 per cent hydrochloric acid to the 100 cc. of solvent first obtained and evaporating directly. The results, however, were always slightly low.

The method for the powdered material seemed to be pretty well in hand by this time. It was believed, however, that it would be necessary to grind only the penetrated samples as the earlier work on coated barley gave good results on whole grains. However, in view of the fact that several modifications had been introduced into the method during the work on ground samples, another lot of coated poisoned barley was made up containing 0.248 per cent of strychnine. Analysis of the whole grains gave 0.234 per cent. After drying and grinding, analysis gave 0.244 per cent on the original moisture basis. This indicated that the samples should be ground whether coated or penetrated.

During the progress of the investigation, several plants manufacturing poisoned grains were visited, and their methods were carefully observed. The weights of the ingredients of the batches of poison material were checked up, and samples of the finished products were taken. These were subsequently analyzed with the following results:

PLANT NO.	SAMPLE NO.	TOTAL WEIGHT pounds	STRYCHNINE WEIGHT	STRYCHNINE per cent	FOUND per cent
1	1	1106	3.5 lbs.	0.316	0.318
2	1	237.5	11 oz.	0.289	0.281
3	1	133	5 oz.	0.235	0.235
	2	130	5 oz.	0.244	0.244
	3	135	5 oz.	0.231	0.233
	4	131	5 oz.	0.239	0.246

The method of analysis as finally worked out is as follows:

#### METHOD FOR STRYCHNINE IN POISON BARLEY.

If the sample is too moist to grind well, dry at 50°–60°C. overnight. Grind finely and weigh 25 grams into a dry 300 cc. Erlenmeyer flask. Add (conveniently at 3 p. m.) 120

cc. of ether-chloroform mixture (2 : 1) and stopper tightly. Allow to stand 30 minutes with occasional agitation. Add 25 cc. of 10 per cent ammonium hydroxide. (For wheat use 15 cc.) Shake 1 hour and allow to stand overnight. In the morning shake again for 15 minutes. Add approximately 5 cc. of sirup, such as Karo, to clarify the solution somewhat and shake again for 15 minutes. Pour off 100 cc. of the solvent and transfer to a separatory funnel. Add enough ether to cause the solvent layer to rise to the top in the subsequent extractions. Extract once with 50 cc. of 0.5 per cent hydrochloric acid and six times with 25 cc. portions, receiving them into a 400 cc. beaker. (A milky emulsion will be formed in the shaking but this should be entirely run off each time.) Evaporate the combined extracts to 50 cc. Cool, and make alkaline with ammonium hydroxide, avoiding any excess. Make slightly acid with acetic acid and warm gently for a few minutes, whereupon a flocculation of the suspended matter will take place. Cool, add 2 cc. of a 10 per cent solution of neutral lead acetate and transfer to a 100 cc. volumetric flask. Make to volume, shake, and filter into a dry, graduated, 100 cc. glass-stoppered cylinder without washing and note the volume obtained. Add 3 cc. of a 3.0 per cent sodium oxalate solution. Shake, and allow to stand for 15 minutes; filter into a dry, graduated, 100 cc. cylinder; and note the volume obtained. Transfer to a 250 cc. beaker, evaporate to 70 cc., and cool. Add 25 cc. of a recently filtered saturated picric acid solution and allow to stand 3 hours with occasional stirring during the first half hour. Filter on a tared Gooch crucible and wash with 50-80 cc. of cold water. Dry at 105°C. and weigh. Strychnine picrate  $\times 0.5932$  = strychnine.

Calculate the weight of the sample from the record of the volumes concerned in the several processes and then obtain the percentage of alkaloid in the usual manner.

An example of the calculation is as follows:

Loss at 60°, 7.12 per cent;  $100 - 7.12 = 92.88$  per cent residue.

The material is ground, and 25 grams is taken for analysis. Volume of solvent = 120 cc. One hundred cc. of this material is drawn off, purified, made up to 100 cc., filtered, and 96.9 cc. of filtrate obtained. Three cc. of sodium oxalate solution is then added, and the solution is filtered again, 97.0 cc. filtrate being obtained. The weight of dried material represented by this last filtrate is therefore:

$$\frac{97}{99.9} \times \frac{96.9}{120} \times 25 = 19.6 \text{ grams.}$$

The percentage of strychnine found in the dry material multiplied by the above factor,  $\frac{92.88}{100}$ , gives the percentage on the original moisture basis. In this determination 0.0828 gram of strychnine picrate was obtained; therefore—

$$\frac{0.0828 \times 0.5932 \times 92.88}{19.6} = 0.233 \text{ per cent strychnine.}$$





CHARLES DAYTON WOODS, 1856-1925

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## CHARLES DAYTON WOODS

Dr. Charles D. Woods, an active member of the Association of Official Agricultural Chemists for a number of years, died at his home in Newton, Mass., March 30, 1925.

Dr. Woods was born in Brooks, Maine, September 11, 1856. He was the son of Henry J. and Maria (Colcord) Woods. When Charles was a lad in his early teens the family moved to Newton, where the elder Woods engaged in business and lived, with the exception of a few years spent in Orono, until his death a few years ago. The aged mother was still living at the time of Dr. Woods' death, and he is also survived by his wife, Mary Morgan Woods, whom he married in 1882, and by two sons, Harry D. and Dr. William C.

Like most boys of his time, his early education was obtained in the public schools. In 1876 he entered Wesleyan University and was graduated with the class of 1880, receiving a degree of B. S. for work in the sciences. He specialized in chemistry under Dr. W. O. Atwater, who at that time was working on human foods and was an enthusiast on the subject. This was the beginning of the extensive food work carried on by Atwater and Woods in the following years.

After graduation Woods remained at Wesleyan for three years as assistant chemist. It was during this period, 1881-1883, that he became actively interested in the problem of the sources of the nitrogen of plants. Atwater, in his article "Acquisition of Atmospheric Nitrogen", published early in 1885 in the *American Chemical Journal*, expresses obligations to Woods for the faithful and skillful performance of the details of the experimental work upon which the article was based. The first part of this work was done in 1881, and the results were reported briefly by Atwater at the meeting of the American Association for the Advancement of Science that year. A second series of experiments, essentially a duplication of that conducted in 1881, was performed in 1882, and both afforded what is perhaps the first positive evidence of the acquisition of large quantities of nitrogen from the air by certain plants during their period of growth. Peas were used in these experiments. Whether the nitrogen obtained by them from the air was free or combined was not conclusively proved at this time, but the quantities acquired were in many instances so large as to leave little doubt that the peas were in some way utilizing the free nitrogen of the air.

From 1883-1888 Woods was employed as a teacher of the sciences at Wilbraham Academy, Wilbraham, Mass. In 1888, after the passage of the Hatch Act, the Storrs Agricultural Experiment Station was established, and Dr. Atwater was made director and Dr. Woods chemist with offices and chemical laboratories located at Wesleyan University, Middletown, Conn. In 1891 Dr. Woods was made vice-director and chemist, which position he filled until 1896. In the eight years he was connected with the Storrs Station he was engaged continuously in the study of problems important to agriculture and in connection with the food of man. The



question of the utilization of the free nitrogen of the air by plants continued to be of great agricultural and scientific interest. European investigators were vigorously pursuing studies along this line, and Atwater and Woods resumed their work at the Storrs Station. Reports of very extensive and carefully conducted investigations by them are included in the Station reports for 1889 and later years.

In glancing through the Storrs reports for the years 1888-1892, other publications on problems studied and reported by Woods are to be found. Such, for example, are: "Roots of Plants as Manure", in the report for 1888; "Effects of Different Fertilizers Upon the Composition of Corn", in the report for 1889; "Fertilizing Ingredients in Crop and in Roots of Legumes", in the report for 1890; and "Effect of Nitrogenous Fertilizers Upon the Percentages of Protein in Grasses and Grain", in the report for 1892. In the reports for 1893, 1894, and 1895 are found studies of rations for milch cows, experiments in feeding sheep, and digestion experiments with sheep.

Aside from the purely agricultural work, Dr. Woods was closely associated with Dr. Atwater in his extensive investigations on the food of man. All the analytical work necessary in connection with their investigations, the results of which fill many pages of the Storrs reports, were in his charge, and many of the dietary studies which were carried on with people in various walks of life were planned and carried out under his supervision. The mass of data obtained by Atwater and Woods in their investigations of human foods furnishes the knowledge now utilized continually by many dietitians. The Storrs Station was the first to publish caloric values of foods and feeding stuffs in the United States.

While with Dr. Atwater, Dr. Woods assisted in developing an efficient bomb calorimeter, by means of which the energy value of foods is obtained. He was also closely associated with the construction of probably the best type of respiration calorimeter that had been devised at that time.

In 1896 Dr. Woods was called to the University of Maine, as professor of agriculture and director of the Maine Agricultural Experiment Station, a position which his previous training at Storrs had admirably fitted him to fill. He continued in the dual position until 1904 when he resigned the professorship to devote his entire time to the work of the Experiment Station. Under his direction the Station prospered and the scope of its work was considerably increased, but in this brief memorial it is only possible to mention a few of the many things he accomplished during the nearly twenty-five years of his directorship.

In reviewing the Maine Station reports it is found that the studies relating to human food, in which he had become so much interested at the Storrs Station, were continued in Maine. In the report for 1898 important digestion experiments with human subjects are given. In the report for 1899 an extensive study of nuts as food and of cereal breakfast foods, which attracted wide-spread attention, are reported.

Dr. Woods acted as food expert to the U. S. Department of Agriculture 1894 to 1908, when the Federal appropriation for this purpose was discontinued. Under his direction the growth of the Station was rapid; the working staff was nearly doubled in a few years, and the Station building was enlarged to make room for several much needed laboratories and offices. Two experimental farms were acquired, one in the potato growing region and the other in the dairy and orchard section of the state.

In 1897 he was instrumental in getting a feeding stuffs inspection law passed by the Maine legislature and later a law for the inspection of agricultural seeds. Likewise, in 1905, he took a prominent part in obtaining the Maine pure food and drug inspection law, of which he was made the executive for several years. In this position he was highly regarded by the food and drug officials of the country, and he took a prominent part in their activities, occupying many positions of honor.

Dr. Woods was a firm believer in scientific research as the best means of solving most agricultural problems; therefore, under his direction the work of the Agricultural Experiment Station, Orono, Maine, was largely of an investigational nature. He had little use for the so-called popular experiments. He believed in handling a few subjects well rather than attempting to cover a large field of inquiry.

The criticism that was sometimes made that the Maine bulletins were too technical or scientific for the average reader was probably true in some cases, but in lieu of issuing a more popular bulletin for the general reader, Dr. Woods wrote weekly, for several years, what was known as a "publicity letter" on some timely agricultural topic. These letters were published by most of the daily and weekly papers in the state and together make quite a volume.

After leaving Maine Dr. Woods was called to Camp Devens in 1921 to act as consultant in agriculture to the U. S. War Department and later to a position as technical adviser in agricultural science for the State of Massachusetts. He held the latter position until a few weeks before his death. Therefore, practically his whole life was devoted to the improvement of agriculture. His work was regarded highly by his co-workers in similar institutions. As a member of the Association of Land Grant Colleges and Agricultural Experiment Stations his councils and opinions were considered with confidence and he occupied many positions of honor.

Dr. Woods was a strong man with a sterling character, but he held very positive convictions; he was outspoken and somewhat blunt in his remarks at times but at heart he was kind, generous, and sympathetic. He was best liked by those who knew him best. He was a useful citizen, taking an active interest in town and church affairs wherever he lived, and in his death not only agriculture but the country has lost a valuable public servant.

JAMES M. BARTLETT.

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## FIRST DAY

### MONDAY—AFTERNOON SESSION—Continued.

#### REPORT ON DAIRY PRODUCTS.

By JULIUS HORTVET (State Dairy and Food Department, St. Paul, Minn.), *Referee*.

The collaborative work carried on during the past year has been chiefly in connection with a further study of the vacuum method for the determination of moisture in cheese. The results of the work are presented and fully discussed in a separate report submitted by the associate referee assigned to that subject.

#### BUTTER.

During the past two years some correspondence has been carried on with reference to the official methods for analysis of butter, attention being given particularly to the instructions for sampling and preparation of sample. The sampling methods referred to in the correspondence are known respectively as the "trier" method, the "wedge" method, and the "auger" method. The last named method has not been published. A communication received from A. L. Gibson, Guelph, Canada, contains some valuable suggestions relative to improvements in the section dealing with methods for the examination of butter. Two recommendations are offered, one relating to *a method for determining acidity* and the other to *a method for distinguishing butter made from pasteurized cream*. These recommendations are quoted, in part, as follows:

(1) Various methods of estimating acidity are applied, either water and alcohol or an ether-alcohol mixture being used as a solvent. Personally, I believe that a delicate test which would estimate the true rancidity or oxidation of butterfat would be more valuable in distinguishing good from inferior butter. At any rate, a test which will estimate acidity or rancidity of butter seems to be the desire of many dairymen at the present time.

(2) Most of the butter is distinguished by a modification of the Storch test, which requires paraphenylenediamine and hydrogen peroxide as reagents. Since the result of this test means a difference in the price obtained for butter, such a test should be reliable. For this reason I would suggest that a test designed for this purpose be investigated and, if possible, included in the official methods.

In line with suggestions for further collaborative work on methods for butter, attention is directed to the recently published paper by Mitchell and Alfend<sup>1</sup>. This paper gives a full criticism of present methods for the preparation of butter samples for analysis and proposes a new method, which is quoted in full as follows:

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<sup>1</sup> *This Journal*, 1925, 8: 574.

Warm the sample, 250-500 grams, in a closed vessel until about half of it is melted. Stir with a malted milk mixer for 2-3 minutes, with up-and-down movement of the stirring device. The final temperature must be 31°-34°C., at which temperature the butter will completely wet the sides of the container. If the temperature is below 31°C., continue stirring until this temperature is reached. A temperature above 34°C. will indicate that the sample has been warmed too much. In this case, cool the sample until solid and repeat the warming and mixing.

Regarding comments made on the method for preparation of sample<sup>1</sup>, it may be stated that the paragraph was written in an attempt to clarify and improve, so far as seemed expedient, the former method, which had been frequently subjected to criticism. It was believed desirable to harmonize the A. O. A. C. method of preparation of sample with the procedure followed by members of the American Dairy Science Association and the American Association of Creamery Butter Manufacturers. Meantime, it seems necessary that this method be subjected to continued collaborative study and that the form proposed by Mitchell and Alfend be considered as a basis for collaborative work during the ensuing year.

#### CHEESE.

As a result of two years of collaborative studies the associate referee has recommended for final adoption as official a vacuum method for the determination of moisture in cheese. During the past year seven collaborators reported results obtained on 31 samples representing 10 types of product, domestic and foreign. The results and comments submitted by the collaborators justify a recommendation that this method, in the form described by the associate referee, be adopted as official. In a communication under date of August 27, 1925, the associate referee suggests that an amendment be made to the description of the method for preparation of a sample of cheese<sup>2</sup> for analysis by appending the following sentence:

Before starting analysis allow the prepared sample to stand several hours, or preferably overnight, in a tightly closed container.

The purpose of this change is to allow sufficient time for the moisture to become distributed uniformly throughout the sample. A further suggestion relating to paragraphs 2, 30, 42, 53, 67, and 88, which deal with the preparation of samples of various dairy products, reads as follows:

It occurs to me that the stirrer method recommended for butter could be made applicable to all the dairy products with the exception, of course, of malted or dried milk and cheese. The only objection to the method would be that it would probably interfere with the specific gravity determination for milk due to the possible beating of air into the sample by the stirrer.

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 276.

<sup>2</sup> *Ibid.*, 278

The trend toward a unification of methods applied to various classes of products seems to make desirable the recommendation that the referees devote some attention to this phase of the subject during the ensuing year. Vacuum methods of drying seem to be gaining in favor, as may be noted particularly in connection with studies recently conducted by Snyder and Sullivan on methods for determining moisture in flour<sup>1</sup>. Attention is also directed to the contribution by Spencer<sup>2</sup>. It will be profitable to make a study of these investigations with a view to a possible unification of the drying methods applied to a large group of products. In order to provide for collaborative studies on methods of analysis under the headings *Butter* and *Cheese*, it appears necessary that an associate referee be appointed and instructed to deal with these two subjects, and that he devote special attention to methods of sampling and preparation of sample for analysis.

#### MILK AND CREAM.

A. L. Gibson offers the following criticisms regarding the A. O. A. C. method for albumin in milk<sup>3</sup>:

The present method calls for the precipitation of the albumin by heat after the removal of the casein by precipitation with 10 per cent acetic acid or saturated alum solution. This method often gives a low result owing to incomplete coagulation of the albumin by heat. I would suggest a more reliable method by using 10–12 cc. of Almen's reagent to precipitate the albumin in 10 grams of milk. The reagent consists of 4 grams of nitrogen-free tannic acid dissolved in 190 cc. of 50 per cent alcohol plus 8 cc. of 25 per cent acetic acid.

The Gerber method<sup>4</sup> has been used extensively during the past twenty-five years in various European countries for determining the percentage of fat in milk. Some correspondence has been conducted within the past year relative to certain adaptations of this well-known method for use in the determination of fat in numerous products manufactured from milk, such as cheese, cream, butter, condensed milk, dried milk, and ice cream. While it is universally granted that the method is capable of giving good results, especially when applied to milk, numerous criticisms have appeared, beginning as early as 1903, or possibly earlier<sup>5</sup>. The method directs that a 11 cc. sample be treated with a mixture of 10 cc. of sulfuric acid and 1 cc. of amyl alcohol. Much difficulty has been encountered owing to variations in the quality of commercial grades of amyl alcohol. Attention should also be called to the fact that modifications of the Babcock test, in which the addition of amyl alcohol, acetic acid, or other reagent is directed, have been subjected to collaborative

<sup>1</sup> *J. Ind. Eng. Chem.*, 1924, 16: 741, 1163; 1925, 17: 311.

<sup>2</sup> *This Journal*, 1925, 8: 301.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 260.

<sup>4</sup> *Milch-Ztg.*, 1892, 21: 891; 1893, 22: 363; 1895, 24: 169.

<sup>5</sup> *Analyst*, 1903, 28: 213; 1904, 29: 113; 1905, 30: 326; 1925, 50: 413.

studies by this association, and as a result further consideration of these tests has been discontinued. This subject was discussed by the referee on dairy products in his 1917 report<sup>1</sup>. The criticisms relative to modified Babcock tests are equally applicable to the Gerber method and its various adaptations to dairy products other than milk. By way of confirmation of these criticisms there is submitted Table 1, which includes results obtained during the past year by O. Kueffner, assistant chemist in the Minnesota Dairy and Food Department.

TABLE 1.

*Results of fat determinations by different methods and refractive index at 40°C.*

SAMPLE NO.	PERCENTAGE			REFRACTIVE INDEX AT 40°C.		
	ROESE-GOTTLIEB	BABCOCK	GERBER	ROESE-GOTTLIEB	BABCOCK	GERBER
BUTTER.						
359	79.36	79.0*	81.77	.....	.....	1.4530
381	80.42	80.7*	84.67	.....	.....	.....
			83.65			
349	81.32	81.5*	84.66	.....	.....	1.4530
CREAM.						
542	21.09	21.0	.....	.....	.....	.....
546	20.62	20.6	20.4	1.4550	1.4545	1.4538
560	25.98	26.0	25.6	1.4550	1.4550	1.4538
290	33.78	33.8	33.0	1.4552	.....	1.4535
Sour†	25.03	26.0	21.4	1.4542	1.4540	1.4530
Sour†	34.86	35.0	28.9	1.4552	1.4550	1.4529
ICE CREAM.						
54	23.08	.....	22.9	1.4532	...	1.4522
53	12.58	.....	12.4	1.4535	...	1.4530
57	12.12	.....	11.4	1.4540	.....	1.4530
63	12.33	.....	11.9	1.4540	.....	1.4532
62	14.46	.....	13.9	1.4542	.....	1.4532
407	12.57	.....	11.92	.....	.....	.....
126	13.45	.....	12.88	.....	.....	.....
125	12.07	...	11.10	1.4540	.....	1.4530

\* Tests made with Hortvet butter-test bottle.

Refractive index range of milk fat at 40°C. .... 1.4538-1.4552

Refractive index of amyl alcohol. .... 1.3983

† Acidity 0.64 per cent and 0.68 per cent, respectively.

The results given in Table 1 are significant. It is apparent that the fat separated in the Gerber tests was contaminated with a foreign substance. The odor of the separated fat, especially in the tests made on the samples of butter, was strongly indicative of amyl alcohol. The low refractive index figures confirm the presence of an adulterant, presumably amyl alcohol, or some compound formed during the operation

<sup>1</sup> *This Journal*, 1917, 2: 238.

of the test. On the other hand, it will be noted that normal refractive index figures were obtained on the fat separated in the Babcock and Roese-Gottlieb procedures. These figures are well within the range for pure milk fat. Especially significant are the very low results obtained on the samples of sour cream and also the results on the ice cream samples, which are in general rather low in comparison with the Roese-Gottlieb figures. Two papers dealing with this subject, recently published by Fisher and Walts<sup>1</sup>, discuss various methods applicable to ice cream and include complete tabulations of results by the various methods subjected to comparative study.

A few inquiries have been received relative to the relationship between the official Roese-Gottlieb method and the well-known Mojonnier procedure. In reply to these inquiries the statement has been made that the directions for determining fat in dairy products by means of the Mojonnier apparatus constitute, essentially, the details of the Roese-Gottlieb procedure. The use of the term "Mojonnier" as descriptive of a distinct method rests on a misconception. The term relates specifically to an apparatus or machine designed especially for the purpose of shortening the time required for carrying out a determination. It is commonly known that the apparatus is highly efficient for the purposes for which it is intended. The method employed, however, is nothing more nor less than the standard Roese-Gottlieb. Table 2 is submitted to illustrate how closely the results obtained by the procedure described in

TABLE 2.

*Official Roese-Gottlieb results compared with Mojonnier machine results.*

COLLABORATOR	SWEETENED CONDENSED MILK		EVAPORATED MILK	
	Official R-G Method	Mojonnier Apparatus	Official R-G Method	Mojonnier Apparatus
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Wisconsin Condensed Milk Co. Burlington, Wis.	9.672	9.658	7.876	7.882
	9.680	9.664	7.878	7.892
Borden's Condensed Milk Co. New York, N. Y.	9.74	9.72	7.92	7.91
	9.72	9.72	7.91	7.92
	9.73	. .	. . .	7.91
State Dairy and Food Dept. St. Paul, Minn.	9.75	9.62	7.91	7.90
	9.72	9.64	7.95	7.89
	9.73	....	7.95	....
Carnation Milk Co. Oconomowoc, Wis.	. . .	....	7.86	7.86
Mojonnier Bros. Chicago, Ill.	. . .	9.687	..	7.888
		9.683		7.891

<sup>1</sup> *J. Dairy Sci.*, 1925, 8: 54, 196.



*Methods of Analysis*, A. O. A. C., 1925, 62, 16, and referred to in sections following, compare with results obtained with the Mojonnier machine. These results are compiled from the referee reports of 1915-1917.

During the past year a number of authentic samples of cream have been collected for the purpose of an investigation of their cryoscopic properties. The results obtained on these samples are included in Table 3.

TABLE 3.  
*Cryoscopic results obtained on samples of cream.*

LABORATORY NUMBER	FAT	TOTAL SOLIDS	SOLIDS NOT FAT	ACIDITY AS LACTIC	FREEZING POINT — 0°C.
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
1714	37.0	42.70	5.70	0.10	0.560
1716	22.5	30.48	7.98	0.12	0.549
1739	25.5	32.39	6.89	0.13	0.549
1756	20.5	28.17	7.67	0.12	0.553
1757	23.0	30.38	7.30	0.12	0.554
1760	23.0	30.38	7.38	0.12	0.545
1944	17.5	24.69	7.19	0.08	0.542
2270	27.0	33.56	6.56	0.11	0.547

The figures in Table 3 are confirmatory of some miscellaneous results recorded in connection with samples received at the Minnesota Dairy and Food Laboratory during recent years and warrant the conclusion that normal sweet cream yields freezing-point values fairly within the range for normal milk. From a practical standpoint the freezing-point test is useful, especially in detecting water added fraudulently or for the purpose of "standardizing" to the legal limit. The customary practice is, presumably, to mix high-test cream with skimmed milk, but in certain instances there may exist the more reprehensible practice of diluting with water. Experience with the application of the freezing-point test to authentic samples seems to justify a recommendation that the cryoscopic method<sup>1</sup> be adopted as official for cream.

#### MALTED MILK AND DRIED MILK.

During the past year a study has been made of methods for the determination of moisture in dried milk. A brief report has been submitted by the associate referee, who concludes with the statement: "Further work will be necessary before any definite recommendation can be made".

In addition to the collaborative studies recommended at the meeting held a year ago, particularly with reference to methods for the determination of fat and moisture in dried milk, it seems desirable that atten-

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 265.

tion be directed to recent information submitted to the Joint Committee on Food Definitions and Standards relative to the product known as malted milk. The committee has felt justified in undertaking an investigation with a view to a revision of the present definition and standard for this product. It will therefore be required that methods applicable to this group of products be perfected and extended. The situation appears to indicate a demand that the associate referee devote his activities during the ensuing year to an intensive study of these methods.

#### ICE CREAM.

The Associate Referee on Ice Cream has not submitted a report, owing to the fact that he was appointed at a rather late date during the past summer. The referee appointed a year ago was obliged to resign. A letter received from A. C. Dahlberg, under date of September 15, contains the following statement:

I regret that it has not been possible for me to begin work on the official methods of analyzing ice cream. Rather than submit an impromptu report I think it advisable to send in no report whatever for this year. During the late fall I will be in a position to actively start this work. At the present time I am completing work for the report on the Gerber and Babcock tests for the American Dairy Science Association, and this work will require my full time for the balance of this month.

#### RECOMMENDATIONS<sup>1</sup>.

In concluding this report, the following recommendations are made:

(1) That an associate referee be assigned to the subject of butter and that particular attention be given to the following points:

- (a) method of sampling,
- (b) preparation of sample,
- (c) method of determining acidity, and
- (d) method of distinguishing butter made from pasteurized cream.

(2) That the vacuum method recommended by the Associate Referee on Cheese as a result of collaborative studies during the past two years be made official (final action).

(3) That the referee give attention to the proposed change in the method for determining albumin in milk.

(4) That the cryoscopic method be adopted as an official method applicable to cream (first reading).

(5) That the associate referee assigned to malted milk and dried milk be instructed to devote attention to the following subjects, with reference particularly to malted milk:

- (a) method for fat,

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 79.

- (b) method for moisture,
- (c) determination of cold water extract, and
- (d) determination of carbohydrate.

(6) That the Associate Referee on Methods for Ice Cream be instructed to follow the plans for collaborative study so far formulated, with the suggestion that special attention be given to methods for the determination of sugars, milk solids, and gelatin.

## REPORT ON THE DETERMINATION OF MOISTURE IN CHEESE.

By LLOYD C. MITCHELL (U. S. Food and Drug Inspection Station, St. Louis, Mo.), *Associate Referee*.

This year's work was a continuation of the study made last year of the present tentative A. O. A. C. method<sup>1</sup> and the proposed vacuum method<sup>2</sup>. One of the collaborators also reported some results obtained by the distillation method, as modified by Bidwell and Sterling<sup>3</sup>.

The results of this study were contributed by the following collaborators, to whom the referee at this time desires to express his thanks: O. L. Evenson, Bureau of Chemistry, Washington, D. C.; Carlos A. Greenleaf and John L. Heid, U. S. Food and Drug Inspection, Cincinnati, Ohio; O. S. Keener, U. S. Food and Drug Inspection, St. Louis, Mo.; J. T. Keister, Bureau of Chemistry, Washington, D. C.; T. O. Kellems and J. C. Palmer, U. S. Food and Drug Inspection, San Francisco, Calif.; C. A. Roach, U. S. Food and Drug Inspection, Chicago, Ill.; and Miss Dorothy B. Scott, U. S. Food and Drug Inspection, New York, N. Y. Their results are given in Tables 1, 2, 3, and 4.

## COMMENTS OF COLLABORATORS.

*Evenson:* For the tentative method I used a Freas electric oven and kept the temperature at 98°–100°C. The samples were placed on a shelf near the center of the oven and grouped around the bulb of the thermometer. Four to five weighings were necessary. Sand was used. For the vacuum method also a Freas electric oven was used, and the temperature was kept as near to 100°C. as possible. The pressure in general varied from less than 10 to 30 mm. of mercury. The samples were partly dried on a steam bath before they were placed in the oven; with 60 per cent of them, 3 hours' drying was found to be sufficient. None was dried more than 4 hours.

*Greenleaf and Heid:* The proposed vacuum moisture method appears to have two advantages: it gives closer checks and avoids the repeated weighings necessary when steam oven drying is used.

*Keener:* The vacuum method is more convenient, and more uniform results are secured by it than by the tentative method. The distillation method apparently yields less water than the vacuum method, and it is difficult to read the percentages in less than 0.25 per cent.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 278.

<sup>2</sup> *This Journal*, 1925, 8: 477.

<sup>3</sup> *Ibid.*, 295.

TABLE 1.  
Results of determination of moisture in cheese by two methods.

ANALYST	TYPE OF CHEESE	METHOD		
		Tentative A. O. A. C.	Vacuum	Distillation
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Evenson	Parmesan	33.83	34.04	
		33.77	34.16	
		33.84	34.14	
		33.76	33.81	
		33.67	33.88	
		33.62	33.95	
		33.57	33.73	
		33.68	33.67	
		33.78	33.61	
		33.69	33.65	
		33.68	33.75	
Greenleaf	Italian	20.25	20.83	
		20.19	20.73	
	Pecorino	27.46	28.14	
		27.38	27.94	
Heid	Italian	29.30	29.80	
	Swiss	34.70	34.90	
	Italian	29.30	30.25	
Keener	Parmesan	31.47	32.35	32.00
		31.73	32.33	31.75
	Canestrato	33.14	33.75	31.75
		33.11	33.71	32.00
	Caciocavallo	40.96	40.89	39.50
		40.59	41.08	39.75
	Provincial	31.45	32.17	31.00
		31.28	32.05	30.75
	Swiss	36.12	38.13	36.50
		36.54	38.01	37.00
Scott	Roquefort	32.95	32.59	
		32.95	32.96	
		38.08	38.10	
		37.98	38.24	
		39.80	40.11	
		39.84	40.02	

*Keister:* The conclusion to be drawn from these results seems to be that in case of soft cheeses with high moisture there is no advantage in the vacuum method, but in the case of hard, dry cheeses, the vacuum method seems to be necessary to drive off the moisture completely. As I recall, results obtained last year show that there was a wider difference found between the two methods than is shown in results here reported. I used 26-27 inch vacuum in every case and used sand as an absorbent.

*Kellems and Palmer:* The sample in each case was prepared by grinding three times through a sausage grinder; it was then placed in a Mason jar, and duplicate portions were immediately weighed out. For the A. O. A. C. method the sample was dried in a platinum dish 9 cm. wide and 2.5 cm. high, containing 3-5 grams of asbestos. The oven was of the water-jacketed type, measured 10 x 10 x 8 inches, and was heated by one or two Bunsen burners. The temperature in the oven varies between 97° and 99.5°C. when the water in the jacket is boiling. For the proposed vacuum oven method the sample was dried in an aluminum dish 6 cm. wide and 1.5 cm. high, fitted with a slip-in lid. The oven was water-jacketed and heated by two pipe burners, one on each side, extending the full length of the oven. The temperature in the oven when the water is boiling is about 98°-99°C.

We wish to emphasize the fact that the time required for drying cheese depends to a great extent upon the fineness of the sample. The sample of Brie cheese reported in Table 4 required over 9 hours and the first sample of Emmenthaler, 10 hours. This was because these samples were placed in the dishes in the same condition that they came from the grinder, that is in strands about the size of vermicelli. The time required

TABLE 2.

*Results of determination of moisture in cheese by two methods and drying for varying periods.*

ANALYST	TYPE OF CHEESE	METHOD				
		Tentative A. O. A. C.			Vacuum	
		3 hours	4 hours	5 hours	3 hours	4 hours
Roach	American	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
		42.48	42.88	*	43.00	*
		42.62	43.04	*	43.05	*
		37.96	38.44	*	38.90	*
		37.00	37.58	38.04	38.55	*
		36.74	*	*	37.25	*
		36.74	*	*	37.10	*
		43.00	*	*	42.25	*
		42.74	*	*	42.30	*
		42.35	*	*	41.25	*
		41.98	*	*	41.50	*
		43.40	43.50	*	42.85	43.35
		43.06	43.20	*	43.20	43.25
		34.94	35.08	*	34.20	34.80
		34.84	34.84	*	34.75	35.10
		37.72	37.98	*	37.45	37.95
		37.88	37.94	*	37.55	37.90
		38.10	38.16	*	37.45	38.00
		37.98	37.98	*	37.35	37.80
		36.14	36.74	37.36	38.00	*
		36.70	37.16	37.54	38.15	*

\* Gain in weight.

TABLE 3.

*Results of determination of moisture in cheese by two methods and drying for varying periods in a vacuum at 97°C.*

ANALYST	TYPE OF CHEESE	METHOD					
		Tentative A. O. A. C.	Vacuum at 97°C.				
			4 hours	4½ hours	5 hours	5½ hours	6 hours
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Keister	Parmesan	33.05	33.87	33.83	33.88		
		33.19	33.90	33.93			
	Pecorino	43.26	43.56				43.71
		43.30	43.55				43.69
	Pecorino	41.57	41.42				41.45
		41.65	41.28				41.43
	Pecorino	33.89	34.60		34.70	34.90	
		33.95	34.64		34.74	34.94	
	Pecorino	32.35	32.38			32.43	
		32.39	32.43			32.41	
	Roquefort	45.47	44.91		45.51		
		45.64	44.95		45.63		
	Cream	51.28	51.13		51.23	51.15	
			51.21		51.06	51.26	

for drying the other samples was not exactly determined, but was from 3½-4½ hours.

Some cheese, too soft to be grated, must be ground in a sausage machine. When so ground or when coarsely grated and weighed into the dish, it should be broken up as fine as possible with a glass rod that has been flattened at the end. We believe that in order to insure complete and rapid drying this caution should be mentioned in the method.

*Roach:* Preference is given to the vacuum method, as it does away with the use of sand or asbestos, and besides it is a somewhat shorter method.

*Scott.* It will be noted that the moisture results in two of the samples are slightly higher when the vacuum was used. Better checks on duplicate samples were obtained when asbestos was used. The vacuum ranged from 28-29 inches.

#### DISCUSSION AND CONCLUSION.

One hundred eighty-one moisture determinations were made on forty samples, representing fifteen different types of cheeses and including both domestic and foreign production. Five of the collaborators reported considerably higher moisture results obtained by the vacuum method on all samples analyzed than by the tentative method; three found results, when averaged, that were practically the same for the two methods; and one showed results, when averaged, that were higher by the vacuum method for hard cheeses, but practically the same for the soft types. The average variation among duplicate results was in favor of the vacuum

TABLE 4.

*Results of determination of moisture in cheese by tentative A. O. A. C. and proposed vacuum methods.*

ANALYST	TYPE OF CHEESE	METHOD	
		Tentative A. O. A. C.	Proposed Vacuum
Kellems	Camembert	<i>per cent</i>	<i>per cent</i>
		53.67	53.92
		54.02	54.31
	Brie	54.55	54.86
		54.76	54.70
	Swiss	34.75	35.37
		34.67	35.46
	Emmenthaler	34.90	35.53
		34.97	35.41
	Emmenthaler	38.73	39.08
		38.83	38.99
	Edam	38.31	37.90
		38.53	37.80
Palmer	Koboho	32.99	32.23
		33.23	32.18
	Canestrato	30.93	31.34
		30.94	31.32
	Caciocavallo	34.14	34.61
		34.03	34.64

method. Twenty-eight of the forty samples gave higher values for moisture by the vacuum method, with an average increase for all determinations when using the four-hour drying period for the vacuum method of 0.23 per cent.

No difficulties were reported by the collaborators regarding the vacuum method as modified by last year's experience. Several, however, called attention again to the repeated weighings necessary in order to obtain constant weight when using the tentative method.

It was again found that the highest moisture values for the vacuum method were usually obtained on four hours' drying, although in many instances three hours' drying was sufficient.

It appears that the vacuum method yields moisture values more uniform than those obtained by the present tentative A. O. A. C. method, and usually higher. The vacuum method requires no auxiliary substance, such as sand or asbestos, and it is more convenient in regard to time and effort.

RECOMMENDATION<sup>1</sup>.

It is recommended that the vacuum method for the determination of moisture in cheese be made official. The method has been published.<sup>2</sup>

## REPORT ON MOISTURE IN DRIED MILK.

By J. T. KEISTER (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

During the past year a further study has been made of methods for the determination of moisture in milk powder. The work has consisted of a comparison of methods of drying at 100°C. in a water oven and drying in vacuum (26''-27'') at two different temperatures, viz., 97° and 80° to 85°C. Flat-bottom aluminum dishes with slip-in covers (diameter about 57 mm., height 17 mm.) and samples of from 1 to 1.5 grams were used in all determinations. Dryings were made in 26''-27'' vacuum, with the dish covers completely removed. A slow current of air, which was passed through strong sulfuric acid at the rate of about 2 bubbles per second, was admitted to the oven during drying. The covers were placed on tightly at the end of the drying period. The material was cooled in a desiccator and weighted. The results obtained on 16 samples (12 skimmed and 4 whole milk powders), with notations on the time necessary for complete drying by the vacuum method, are reported in the accompanying table.

Contrary to the findings of Holm<sup>3</sup>, it was soon found that 1 and 2 hour drying periods were not sufficient for complete drying; therefore these short periods were discontinued. The results obtained confirm those obtained from previous experiments, to the effect that drying under atmospheric pressure cannot be applied in determining moisture in dried milk. The results reported indicate that in some cases all the moisture may be removed by drying in a vacuum at temperatures below 100°C., but in other cases such temperature seems necessary. This conclusion is also at variance with that of Holm. A darkening that occurred when the sample was dried in a vacuum near 100°C. was considerably less when the sample was dried at 80°-85°C. Notable differences, observable among the various samples in the time required for complete drying, are not accounted for by the removal of the fat. The method of manufacture, in all probability, plays a part in the avidity of the product for moisture.

The general conclusion reached from these results is that drying at a temperature near 100°C. for 3-4 hours in a vacuum appears necessary in every case to insure the complete removal of moisture from dry milk.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 80.

<sup>2</sup> *Ibid.*, 44.

<sup>3</sup> *Ibid.*, 1922, 5: 511.



*Moisture in*

SAMPLE NO.	DRYING IN WATER OVEN AT 100°C.	DRYING IN VACUUM OVEN AT 97°C.					
		1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
A	3.06 3.05	4.15 4.08	4.40 4.41		4.53 4.45		4.42
I	5.45 5.41	5.42 5.43		5.497 5.525		5.55 5.54	5.60
II	4.19 4.20	4.06 4.08		4.14 4.17		4.168 4.21	4.28
III	3.08 3.12	3.34 3.31			3.32 3.34	3.39 3.40	3.388
IV	3.43 3.44		3.60 3.63		3.64 3.59		3.62 3.63
V	2.62 2.69		2.96 2.97	2.907 2.95	2.967 2.97	2.996 3.01	
VI	2.48 2.50			2.62 2.679			
VII	2.427 2.465			2.55 2.51			
VIII	2.00 2.09			2.927 2.878	2.966 3.00		
IX	3.68 3.73			5.46 5.42	5.397 5.43		
X	3.47 3.49			3.805 3.836			
XI	4.38 4.44			4.84 4.87			
XII	5.246 5.37			5.76 5.60		5.70 5.74-5.77	
XIII	3.09 3.05			5.759 5.736			
XIV	5.07 5.15			4.88 4.85-4.90	4.92 4.90-4.83		
XV	4.37 4.41			4.03 4.04	4.01 4.06-4.00		
XVI	3.49 3.51			2.91 2.895-2.96	3.44 3.46-3.47	3.50	

*milk powder.*

DRYING IN VACUUM OVEN AT 80°-85°C.			REMARKS
3 hours	4 hours	5 hours	
<i>per cent</i> 3.857	<i>per cent</i> 4.058	<i>per cent</i>	Drying in vacuum at 80°-85°C. did not effect complete drying.
5.57	5.60 5.59	5.48 5.516	3 hours drying at 80°-85°C. seemed sufficient.
4.17	4.216		do
4.26	4.206		
	3.278 3.27	3.35 3.34	Drying at 80°-85°C. for 5 hours necessary.
	3.595 3.647	3.55	Drying at 80°-85°C. for 4 hours necessary.
	2.96 2.854		do
	2.34 2.39		Drying in vacuum at 97°C. seemed necessary.
		2.79 2.70	Not enough results obtained.
		2.89 2.88	80°-85°C. not sufficient to effect complete drying in 5 hours.
	3.69	3.73 3.736	97°C. in vacuum apparently necessary.
	2.81	3.44	do
4.57		4.62	do
4.56			
4.448			3 hours at 97°C. required.
4.55			
2.80	3.01	2.91	do
2.92			
4.98	4.91 4.93	5.07 5.14	3 hours at 80°-85°C. sufficient.
3.78	3.71 3.78	3.99 3.88	3 hours at 97°C. apparently necessary.
	2.97		4 hours at 97°C. apparently necessary.

Incomplete results have been obtained in connection with a few of the samples reported owing to insufficient time for the work. Further work will be necessary before any definite recommendation can be made.

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No report on ice cream was made by the associate referee.

## ON THE DETERMINATION OF CASEIN IN MILK.

By H. C. WATERMAN (Bureau of Chemistry, Washington, D. C.).

The present official Method I<sup>1</sup> for casein in milk appears to have been proposed in 1893 by L. L. Van Slyke<sup>2</sup>. In 1903, at the twentieth annual convention of this association, Van Slyke called attention to the fact that a loss was caused by the re-solution of a part of the precipitated casein, usually about 0.1 per cent, in the excess of acid used<sup>3</sup>. He suggested at that time that the quantity of 10 per cent acetic acid used for the precipitation should probably be reduced from 1.5 cc. to 1.0 cc. or less, reserving judgment as to the precise quantity most suitable for the purpose until the next year's report. No further mention of this matter is to be found, however, in the proceedings of subsequent conventions, and the change suggested by Van Slyke appears never to have been made, since 1.5 cc. of 10 per cent acetic acid for the precipitation of 10 grams of milk plus 90 cc. of water still remains the direction of Method I. Some thirty years, therefore, have passed since any modification of the procedure in question has been adopted by the association.

During this period much study has been devoted to the physical chemistry of proteins, and important facts which bear directly upon the quantitative determination of casein in milk have been brought out. For a time, to be sure, the application in a practicable analytical method of the known facts concerning the precipitation of casein at its isoelectric point was impossible on account of the difficulty and time requirement involved in the determination and adjustment of pH values. The simple and amply accurate indicator methods now available have removed this obstacle, however, and Van Slyke himself<sup>4</sup>, in a paper read before the World's Dairy Congress of 1923, speaks of the isoelectric precipitation of casein as essential in any preparation method designed to yield a pure product. Evidently, if such a precipitation is necessary to the preparation of pure casein, it is also much to be desired in the quantitative determination of the protein.

Fortunately, an approximately isoelectric precipitation could be made with little more trouble than is involved in the use of a fixed quantity

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<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 260.

<sup>2</sup> U. S. Dept. Agr. Div. Chem. Bull. 38, p. 109.

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 67, p. 90.

<sup>4</sup> Proceedings of the World's Dairy Congress, 1923, vol. 2, 1145.

of dilute acetic acid. Even the comparison of the color of an indicator added to the solution to be adjusted with the color of the same indicator added to a standard buffer solution of the pH value desired—a procedure frequently used in the adjustment of hydrogen-ion concentrations by means of indicators—can probably be dispensed with in this case. The titration of a small, accurately measured portion of the milk to be analyzed, rather largely diluted with recently boiled distilled water, to the final color change of an indicator the acid end of whose range falls, under the conditions of the proposed use, between pH 4.6 and pH 4.7 would be very likely to prove sufficient. A larger precipitation aliquot could then be brought to the required pH value by using the indicated quantity of acid and the proportionate dilution with recently boiled distilled water in the absence of any indicator.

There is much to be said, also, in favor of determining the casein nitrogen as the difference between the total nitrogen of the sample and the nitrogen of the filtrate from the casein precipitate. It is well known that the quantitative washing of a protein precipitate is tedious and of doubtful accuracy. Very possibly, therefore, the difference method may prove at once more accurate and more convenient.

It has not been possible, no time having been available for the work, to perfect the details of a method based upon the principles suggested. Such a procedure has been applied with some success, however, to the separation of the milk proteins present in extracts of milk-cacao mixtures—here, the lactalbumin and lactoglobulin are denatured, and so are precipitable at a hydrogen-ion concentration very slightly in excess of that optimal for casein—and this appears at first sight a more difficult case than that of milk unmixed with other proteiniferous material.

This note is presented with the hope that a study may be made leading to the development of a modified method based upon modern practice and accomplishing the purpose of Van Slyke's suggested change.

## REPORT ON FATS AND OILS.

By GEORGE S. JAMIESON (Bureau of Chemistry, Washington, D. C.), *Referee*.

During the past year the study of the determinations of unsaponifiable matter and acetyl value has been in accordance with the recommendations in the previous report<sup>1</sup>.

## DETERMINATION OF UNSAPONIFIABLE MATTER.

The two methods employed were the F. A. C.<sup>2</sup> (method of the Com-

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<sup>1</sup> *This Journal*, 1925, 8: 484.  
*J. Ind. Eng. Chem.*, 1919, 11: 161.

mittee on Analysis of Commercial Fats and Oils of the American Chemical Society), and the Kerr-Sorber, slightly modified.

It was discovered that the Kerr-Sorber procedure as first described<sup>1</sup> gave high results owing to the extraction of substances caused by the hydrolysis of the soap along with the unsaponifiable matter. After much experimentation, it was found that the use of 0.2 *N* potassium hydroxide solution in place of water for the removal of the soap was satisfactory.

Two samples of oils and two samples of commercial fats were distributed for collaborative work.

*Modified Kerr-Sorber Method.*

(As submitted to collaborators.)

Weigh 5 grams of the sample into a 200 cc. Erlenmeyer flask and add 15 cc. of 95 per cent ethyl alcohol. To another 15 cc. portion of alcohol in a flask, add 3 cc. of an aqueous solution of potassium hydroxide (100 grams of alkali in 100 cc. of water). Heat both solutions to boiling. Pour the alkali solution into the flask containing the sample and mix if necessary by gently rotating the flask. Boil gently for 10 minutes, then cool to about 30°C. Add 50 cc. of ether, mix, and transfer to a 500 cc. separatory funnel. Rinse the flask with two successive 50 cc. portions of ether, adding them to the separatory funnel, and mix by gently rotating the funnel. Add 100 cc. of 0.2 *N* solution of potassium hydroxide to the saponification flask, shake, and pour it into the separatory funnel in a slow steady stream. Rotate the funnel very gently to secure better contact of the solutions, *but do not shake*. (Shaking at this stage results in the formation of a stubborn emulsion.) Allow the separatory funnel to stand 5–10 minutes, then draw off the soap solution slowly and as completely as possible. If in any case a layer of emulsion is formed, do not draw it off. Keep the volume of ether at about 150 cc. by replacing that dissolved by the aqueous wash solutions. Treat the ether solution with two successive 100 cc. portions of 0.2 *N* alkali solution in the same manner as described previously. Add 30 cc. of water to the ether and rotate the separatory funnel rapidly to extract the alkali. When the layers have separated, withdraw the water and repeat this treatment until the washings are free from alkali as shown by testing with phenolphthalein. (Usually not more than three washings with 30 cc. portions of water are required.) Transfer the ether solution to a weighed flask, preferably a 300 cc. Erlenmeyer flask, distil the ether, and dry the residue to constant weight. Test as recommended in the F. A. C. procedure.

COMMENTS.

In applying this method to the analysis of various kinds of grease, it has been observed frequently that the addition of 50 cc. of ether to the saponified sample causes the precipitation of a large quantity of soap, particularly if the saponified sample has been allowed to become too cool. However, this has not interfered with the accuracy of the determination. Occasionally a little emulsion is formed in the first treatment with 100 cc. of 0.2 *N* alkali solution. In such cases, the aqueous solution (not the emulsion) is withdrawn after the layers have been allowed to separate for 10 minutes. Rotating for half a minute with a second 100 cc. portion of alkali caused the emulsion to break in most instances.

<sup>1</sup> *Cotton Oil Press*, 1924, 7: 40.

In order to eliminate any possible loss of unsaponifiable matter in distilling the ether, it is preferable to use a suitable spray trap between the flask and the condenser. It is essential to follow the directions as closely as possible. After the collaborative work was under way, a further intensive study of this method was made at the Bureau of Chemistry. The results and the method in a convenient form for use have been published<sup>1</sup>.

*F. A. C. Method.*

The F. A. C. method, as formulated by the referee, has been published<sup>2</sup>, but attention should be given to an additional direction as to rinsing the saponification flask with the first portion of 50 cc. petroleum ether.

The results obtained with both methods are given in Table 1.

TABLE 1.

*Collaborative results on the determination of unsaponifiable matter.*

COLLABORATORS	MODIFIED KERR-SORBER METHOD				F. A. C. METHOD—7 PETROLEUM ETHER EXTRACTIONS			
	1	2	3	4	1	2	3	4
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
J. N. Wurst	1.84	1.40	0.52	0.15	1.90	1.41	0.50	0.15
	1.86	1.44	0.58	0.16	1.88	1.24	0.54	0.18
L. E. Strub	1.97	1.37	0.55	0.17	1.85	1.36	0.54	0.17
	1.85	1.37	0.54	0.18	1.79	1.24	0.52	0.19
J. T. Parsons	1.88	1.30	0.74	0.32	1.78	1.20	0.71	0.22
	1.81	1.21	0.67	0.31	1.79	1.26	0.64	0.26
D. G. Sorber	1.94	1.29	0.60	0.39	2.09	1.37	0.75	0.45
W. F. Baughman	2.00	1.34	0.54	0.20	1.93	1.24		0.17
	1.98	1.29	0.58	0.20	2.00	1.15		0.18
G. S. Jamieson	1.89	1.34	0.55	0.20	1.89	1.19	0.61	0.20
	1.96	1.32	0.55	0.22	1.85	1.17	0.60	0.22
	1.93		0.57	0.20	1.93		0.62	
	1.95							
W. D. Richardson	1.83	1.31	0.61	0.46	1.90	1.22	0.79	0.37
	1.86	1.31	0.63	0.52	1.84	1.27	0.79	0.27
	2.04	1.53	0.81	0.35	1.83	1.28	0.78	0.39
	2.18	1.47	0.76	0.34	1.85	1.35	0.90	0.28
	2.00	1.36	0.74	0.35				
	1.93	1.43	0.64	0.35				

None of the results given in Table 1 has been corrected for titratable acidity in the unsaponifiable matter, although in some instances collaborators included corrected values in their reports. When either method is accurately followed, this correction is unnecessary as it is very small.

<sup>1</sup> *This Journal*, 1925, 8: 439.

<sup>2</sup> *Ibid.*, 1926, 9: 44.

If one procedure only could be made official, the referee would select the F. A. C., because it is the method used in connection with both national and international trading in commercial fat products, but in view of the number of satisfactory results reported by both methods it is recommended that both be made official, with the provision that in the case of the F. A. C. procedure at least seven 50 cc. petroleum ether extractions, as indicated previously, be made in all cases. This practice already prevails in a number of laboratories. With few exceptions, collaborators and others have reported that the Kerr-Sorber method was found to be more rapid than the F. A. C. method and required only the simpler apparatus that is available in any analytical laboratory. On the other hand, other analysts claim that they find that the F. A. C. procedure is as rapid as the Kerr-Sorber and that the apparatus is no more complicated.

Able chemists of commercial firms dealing in fats, etc., have expressed themselves very strongly; they contend that since the F. A. C. method has proved "perfectly satisfactory" after being used for some years both here and abroad, they can see no reason for this association to adopt any other method as official, and would consider such action unfortunate. Although the referee believes that their opinion should receive careful consideration, he can see no objection to the adoption of both methods as recommended, since collaborative study has demonstrated that the F. A. C. method, requiring seven 50 cc. petroleum ether extractions, and the modified Kerr-Sorber method give closely agreeing results. This policy was followed several years ago in the adoption as official of the methods of Hanus and Wijs for the determination of the iodine number.

TABLE 2.

*Results of determinations of acetyl value by two methods.*

COLLABORATORS	A. O. A. C. FILTRATION METHOD				ANDRÉ-COOK METHOD			
	1	2	3	4	1	2	3	4
J. N. Wurst	44.0	59.8	27.6	33.3	34.0	63.5	40.5	21.5
	49.5	65.2	37.3	33.3	26.4	58.1	25.6	15.8
L. E. Strub*	40.0	41.3			50.8	39.7	8.7	7.3
	37.3	43.1			50.4	39.7	9.9	6.1
W. F. Baughman	32.0	51.8	12.0	12.4	28.5	48.5	8.1	9.4
	32.4	51.9	14.6	17.6	28.5	48.4	9.1	10.7
W. D. Richardson†	42.2	48.0	33.4	8.0	32.2	50.5	16.0	10.4
	42.4	49.8	33.2	9.2	30.7	49.6	16.4	10.1
					32.1	50.8	16.5	10.5
					30.7	50.2	17.3	10.8

\* André-Cook results—saponified for 90 instead of 30 minutes.

† Corrected results for filtration. (See comments.)

## ACETYL VALUE.

Four samples were distributed for this study. No. 1 was a mixture of mustard seed oil and castor oil; No. 2, a mixture of crude corn and castor oils; No. 3, a refined cottonseed oil, and No. 4, a crude corn oil.

The results given in Table 2 were reported for the determination of acetyl value by the A. O. A. C. filtration method and the André-Cook method.

## COMMENTS OF COLLABORATORS.

The variation in the results obtained by Wurst by the André-Cook method is due partly, if not entirely, to the manner in which the saponification values were determined. He followed the method and saponified for 30 minutes, whereas the referee knows positively that 60 minutes is sometimes insufficient. A comparison of the time effect was made by Wurst. The results obtained are as follows:

SAMPLE NO.	SAPONIFICATION VALUE	
	30 minutes	90 minutes
1	172.3	175.0
	172.5	175.3
2	186.9	188.5
	184.7	187.9
3	150.2	193.8
	153.6	194.1
	172.5	
	188.5	
4	153.6	190.5
	188.7	190.7

The acetylation of an oil probably is not so simple a process as might be supposed. Wurst acetylated a fresh portion for each determination, while Strub acetylated in one lot enough of each sample for determinations by both methods. This probably accounts for the better checks obtained by Strub. Strub's saponifications were conducted for 90 minutes.

Richardson comments as follows: "On the acetyl value, the attached report, we think, indicates that the A. O. A. C. filtration method is clearly of little value. The André-Cook method, in our hands, in every instance gave results which checked very closely, whereas by the filtration method we find that the results run almost anything. The corrections which you will note in the third column under the filtration method, on the first two results of each sample were obtained as a blank by fol-



lowing the method but using a sample of oil which was not acetylated. We are very much in favor of the André-Cook method on account of its simplicity and the good results obtained by it."

Some of the uncorrected results and Richardson's corrections are as follows:

SAMPLE	ACETYL VALUE—FILTRATION METHOD	
	Uncorrected	Corrected
1	98.8	57.0
	99.0	56.1
	113.9	
2	113.8	65.8
	115.6	65.8
	88.3	
3	88.0	51.8
	87.8	54.4
	63.4	
4	82.6	74.6
	83.7	74.5
	58.8	

#### DISCUSSION.

Without doubt it is difficult to acetylate a given sample so that reasonably agreeing results can always be obtained. The chief difficulty appears to be in the decomposition and complete removal of the excess of active anhydride (as acetic acid) over that required for the acetylation without causing more or less hydrolysis of the acetylated fat. Numerous experimenters have observed that it is not so difficult to determine the acetyl value of castor oil as it is of other oils of radically different composition which are characterized by their lack of hydroxy acids. In analyzing these oils by known methods it has not been possible to show that they contain any hydroxy acids, and it is quite probable that the observed acetyl values are due to the presence in small quantities of other substances such as higher alcohols, mono- or di-glycerides, etc., which can react with acetic anhydride. Since the value of this determination is of chief importance in connection with the examination of castor oil it does not appear necessary at this time to have further concern regarding the difficulties encountered.

As a result of this work and of other studies, it may be concluded that the André-Cook method is not only simpler and quicker than the A. O. A. C. method but that it gives as good results as could be expected in view of the difficulties discussed in connection with the acetylation process. Some attention apparently should be given to securing more data in regard to the proper time for conducting the saponification in view

of Wurst's results. In connection with Richardson's comments on his experience with the filtration method this year, it should be noted that apparently none of the other collaborators encountered this difficulty and yet Richardson's corrected results are in closer agreement with their uncorrected results. In the collaborative work of the previous year also, Richardson reported only uncorrected results, which were somewhat higher (with a few exceptions) than the others reported but not near so high as those of this year.

After careful consideration of the results obtained and of the comments of the collaborators it is recommended that the André-Cook method be further studied with particular reference to the time required for complete saponification.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the F. A. C. method for the determination of unsaponifiable matter, as described in this report, be made official (first reading) and that the present official procedure be withdrawn from the next edition of *Methods of Analysis*.

(2) That the modified Kerr-Sorber method for the determination of unsaponifiable matter be made official (first action).

(3) That the André-Cook method for the determination of acetyl value be studied further with reference to the time necessary for complete saponification and that castor oil or mixtures of castor with other vegetable oils be employed for this work.

(4) That the method of Thomas and Chai Lan Yen<sup>2</sup> for the detection and determination of peanut oil alone or in the presence of other oils be investigated by collaborative study.

#### REPORT ON BAKING POWDER.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Referee*.

During 1925 the A. O. A. C. work on baking powder consisted of further study of the methods for the determination of lead, carbon dioxide, and the neutralizing value of monocalcium phosphate. The determination of fluorine was studied under the direction of J. K. Morton, associate referee. In addition to these studies some preliminary work was done on the development of methods for the separation and estimation of pyrophosphate in the presence of orthophosphate.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9 81.

<sup>2</sup> *J. Am. Chem. Soc.*, 1923, 45: 113.

## ELECTROLYTIC METHOD OF DETERMINING LEAD.

No changes were made in the method as studied last year<sup>1</sup>, but in order to secure more data on the suitability of this method for adoption as an official method, samples of baking powder specially prepared so as to contain approximately 25 parts per million of lead were submitted to collaborators. The collaborative results are shown in Table 1.

TABLE 1.  
*Collaborative results on the determination of lead.*

ANALYST	TIME OF ELECTROLYSIS	LEAD	
		Parts per million	Average
L. D. Mathias Victor Chemical Works Chicago Heights, Ill.	<i>hours</i>	21.15	23.09
		24.35	
		23.43	
		23.43	
A. H. Allen Virginia-Carolina Chemical Co. Richmond, Va.	16	29.4	29.2
	24	29.8	
	24	28.2	
	16	29.4	
	16	29.4	
James K. Morton Bureau of Chemistry Washington, D. C.	18	24.99	26.28
	18	26.92	
	18	26.92	
Milton H. Kemp Calumet Baking Powder Co. Chicago, Ill.		23.1	23.1
		25.6	
		23.1	
		20.5	
Percy O'Meara Department of Agriculture Lansing, Mich.		24.4	24.7
		25.0	
C. C. Albee R. B. Davis Co. Hoboken, N. J.	15	{ 18.2	19.2
		{ 19.1	
	18	{ 19.3	
		{ 19.3	
	20	{ 20.0	
		{ 19.3	
L. H. Bailey	18	24.36	25.0
	18	24.36	
	18	26.92	
	24	24.36	

All the collaborators on lead determination this year reported concordant results. Not only do they check closely for each analyst, but those of different analysts are as concordant as can reasonably be expected in a determination of this sort. As it is believed that the method is a reliable one and capable of producing accurate results, it is recommended that it be adopted as official.

<sup>1</sup> *This Journal*, 1924, 8: 92; 1925, 8: 490.

## GASOMETRIC DETERMINATION OF CARBON DIOXIDE.

This year collaborators were requested to give the dates on which they determined carbon dioxide on their A. O. A. C. samples, the object of this request being to check up on discrepancies in results that might be due to deterioration of the baking powder before some of the determinations were made. The results submitted, however, do not indicate that rapid deterioration had taken place.

Some modifications of the method as adopted have been suggested by collaborators.

*Milton H. Kemp says:*

There is one change in the method which should be made. The reading for temperature and pressure is taken at the beginning of the determination. After the carbon dioxide has been liberated the method states that the apparatus should stand for five minutes to secure equilibrium, and that then the volume of carbon dioxide should be read. It does not state that the temperature when the final reading is taken must be the same as at the beginning. If it is not, it must be made so in order to secure correct results. I have had the temperature go up or down 1° or 2°C., and this variation would make a difference of 0.4–0.8 per cent in the results. This is due to the fact that the air, which was originally in the digestion flask, expands or contracts, as the case may be, making the results too high or too low.

*W. E. Stokes writes:*

The method as proposed, we believe, should not be adopted for the following reasons:

1. Results are inaccurate.
2. Too wide variation in results.
3. Wide variation in results is caused by any slight modification, such as length of time of boiling and method of cooling.
4. The use of a factor weight for temperature and pressure which may change materially during determination.

Stokes then suggests a modification in the method of determining residual carbon dioxide. This modification specifies the use of a 2 gram sample of baking powder and the addition of 3 grams of either sodium chloride or sodium sulfate. The procedure then follows the method up to the point of the final heating. Here he suggests to just bring the solution to a boil and then allow the flask and its contents to cool to room temperature (approximately 1½ hours). Artificial cooling does not insure correct room temperature.

He recommends this modification for the following reasons:

1. Close agreement to available gas as found by other methods.
2. Uniform results.
3. Simplicity.

In support of his contention Stokes submitted results on residual carbon dioxide on the A. O. A. C. sample obtained by two analysts from

his laboratory. When his proposed modification was used, the results varied from 0.220–0.347 per cent, and when the regular method was used they varied from 0.6–1.0 per cent.

#### DISCUSSION.

The referee has given consideration to the comments made by the collaborators, but he can agree with them only in part. The suggestion of using a fixed weight of sample in place of a factor weight and reading temperature and pressure at the time the volume of gas is read is advantageous when working in a room where the temperature is variable, but in order to secure accurate results the temperature and pressure must be read when the volume of gas is measured. If a factor weight is used, temperature and pressure must be read before the sample is weighed. However, in determining residual carbon dioxide so much time elapses before the gas is measured, that there may be such a change in temperature as will produce an inaccurate result. For this reason the referee favors a fixed weight in determining residual carbon dioxide.

The referee, at this time, does not look with favor on the suggestion of adding 3 grams of a neutral salt such as sodium chloride or sodium sulfate to the sample for the determination of the residual carbon dioxide for the following reasons: (1) The method of determining residual carbon dioxide is calculated to follow baking conditions as closely as possible, but the introduction of so large a quantity of a neutral salt would be unlike real baking procedure. (2) The referee tried the addition of 3 grams of sodium chloride to some samples of baking powder and secured results that were not so satisfactory as those obtained from the same samples without the addition of the salt.

In Table 2 are shown the collaborative results on total, residual, and available carbon dioxide. While these results are in fair agreement it is deemed advisable to study again collaboratively this method of determining the gas strength of baking powder. It is suggested that determinations be made by using a factor weight as given in the method and also by using a fixed weight of sample, and reading temperature and pressure at the time that the volume of gas is read.

#### THE NEUTRALIZING VALUE OF MONOCALCIUM PHOSPHATE.

The report of the referee last year recommended "that an attempt be made to secure a method of determining the neutralizing value of monocalcium phosphate that will show the exact quantity of bicarbonate of soda required".

The efficiency of a baking powder, under certain fixed conditions, depends upon the proper proportion of bicarbonate of soda and the acid-reacting material. Hence, in making a monocalcium phosphate baking

TABLE 2.

*Collaborative results on carbon dioxide.*

ANALYST	TOTAL CO <sub>2</sub>	RESIDUAL CO <sub>2</sub>	AVAILABLE CO <sub>2</sub>	DATE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
Percy O'Meara	14.15	0.83		
	14.20	0.78	13.4	4-30-25
		0.80		
C. C. Albee	14.40	0.80		
	14.40	0.80	13.6	4-(p)-25
	14.40	0.80		
J. R. Chittick	14.70			
Jacques Mfg. Co.	14.67			
Chicago, Ill.	14.69			4-(p)-25
	14.62			
	14.65			
Grace Vincent	14.95	0.75	14.20	5-11-25
Royal Baking Powder Co.	14.95	1.00	13.95	to
Brooklyn, N. Y.	14.95	0.60	14.35	5-25-25
	14.95	0.70	14.25	
Wm. G. Warning	14.45	0.8	13.65	
Prov. Chemical Works	14.70	1.1	13.60	4-22-25
St. Louis, Mo.	14.60	1.1	13.50	
Milton H. Kemp	14.88	0.78	14.10	
	14.96	0.70	14.26	4-22-25
	14.96			
J. L. Howerton	14.40	0.40	14.00	
Federal Phosphorus Works	14.42	0.32	14.10	
Anniston, Ala.	14.41	0.20	14.21	4-25-25
	14.37	0.28	14.09	
H. Allen	14.03	0.52	13.51	
	14.03	0.50	13.53	
	14.025	0.50	13.525	6-23-25
	14.025	0.51	13.525	
	14.035	0.52	13.51	
	14.03			
	14.04			
H. L. Moxon	14.045	0.49	13.555	
Virginia-Carolina Chemical Co.	14.045	0.51	13.535	6-26-25
Richmond, Va.				
B. R. Jacobs	14.9	0.9	14.0	
National Cereal Products Lab.	14.8	0.8	14.0	6- 2-25
Washington, D. C.	14.9	0.9	14.0	
L. D. Mathias	14.48	0.67	13.81	
L. H. Bailey	14.6	0.8	13.8	
	14.5	0.8	13.7	5- 4-25
	14.6	0.8	13.8	

powder the manufacturer wants to know how much phosphate to use in any particular mix. All the different methods that have been proposed to make this determination yield varying results. Such a condition leads to confusion and sometimes to disputes in the trade.

The methods now in general use give results that indicate that the phosphate will neutralize more bicarbonate of soda than it actually does. This is shown by making a baking powder according to the proportions indicated by one of these methods and then determining the amount of residual carbon dioxide in the baking powder, or by determining the reaction of the baking powder residue after water and heat have been applied. The samples examined by the referee showed the presence of an excess of sodium bicarbonate.

The referee devised a method for the determination of neutralizing value by making mixtures containing different proportions of bicarbonate of soda and monocalcium phosphate, and then determining the hydrogen-ion concentration of their water solutions. Such a mixture that had a pH of 7 was considered to be exactly neutral. The accuracy of results was checked by determining residual carbon dioxide on these mixtures. Those mixtures which showed a pH of 7 or less had no residual carbon dioxide. The results of this study of the referee have been published<sup>1</sup>.

Samples of monocalcium phosphate were sent to collaborators with the request that they determine the neutralizing value by making a series of baking powders having varying proportions of soda and phosphate

TABLE 3.  
*Collaborative results on neutralizing value.*

ANALYST	NEUTRALIZING VALUE
Percy O'Meara	60.7
W. C. Luckow American Institute of Baking Chicago, Ill.	63.7
G. A. McDonald Victor Chemical Works Chicago, Ill.	66. +
Wm. G. Warning	58.0
F. B. Carpenter Virginia-Carolina Chemical Co. Richmond, Va.	64.0
L. H. Bailey	62.0

<sup>1</sup> *This Journal*, 1925, 8: 444

and determine the pH of their aqueous solutions, that proportion which gave a solution having a pH of 7 being the correct one for a neutral product and the ratio of bicarbonate to phosphate being the neutralizing value of the phosphate in question.

The collaborative results, as shown in Table 3, are not in close agreement. This may be expected, however, with the first trial of any method, and more experience with the method will undoubtedly lead to much closer agreement in results.

The ratio of soda to phosphate obtained by this method is so much lower than that obtained by the titration methods now in use that the proposed method has not met with favor among the trade. It is the practice to make baking powders with considerable excess of soda, and the titration methods now used show up such excess.

#### SEPARATION AND ESTIMATION OF PYROPHOSPHATE.

Since increasing quantities of sodium pyrophosphate are being used as a baking acid and no method for estimating pyrophosphates is now included in *Methods of Analysis*, it seemed desirable that some method be devised. Accordingly, the referee sent to collaborators a mixture of ortho- and pyro-phosphates with the request that they determine the pyrophosphate present and submit in detail the method employed in making the determination. Three collaborators reported and gave their methods. The results are quite encouraging, and it is felt that progress has been made.

##### *Method Submitted by A. H. Fiske, Rumford Chemical Works, Providence, R. I.*

To a 0.5 gram sample, add 100 cc. of distilled water and 10 cc. of dilute acetic acid; boil; and filter if necessary. Add 20 cc. of solution A (Solution A = 10 grams of magnesium chloride + 10 grams of ammonium chloride + 10 grams of ammonium acetate + water to make 100 cc.) and allow to boil on a sand bath for about a minute. Remove the solution from the sand bath and allow to stand overnight. Filter on a Gooch crucible, dry, ignite, and weigh as magnesium pyrophosphate.

##### *Method Submitted by W. E. Stokes, Royal Baking Powder Co., Brooklyn, N. Y.*

(This method is based on the fact that magnesium pyrophosphate is insoluble in hot dilute acetic acid while magnesium orthophosphate is soluble.)

##### *(A) Separation:*

1. Weigh out 10 grams of sample and dissolve it in about 500 cc. of distilled water.
2. Filter, if necessary, into a 1 liter volumetric flask and dilute to volume.
3. Transfer 100 cc. aliquots to 400 cc. beakers and dilute to 200 cc. with distilled water.
4. Acidify with glacial acetic acid, using methyl red indicator, adding the acid until a distinct red color is obtained.
5. Add 50 cc. of magnesium acetate reagent and place the beaker on a hot plate.
6. Heat, with frequent stirring, until the contents of the beaker begin to boil.
7. Transfer to a steam plate and allow to remain there for 2 to 3 hours.
8. Filter through a slow filter paper and wash with hot distilled water containing 10 cc. of magnesium acetate reagent per liter. Reserve the filtrate.



*(B) Estimation of pyrophosphate:*

1. Dissolve the precipitate in (A) 8 back into the same beaker in which the precipitation was made, by washing first with dilute hydrochloric acid (1 + 10) and finally with distilled water.

2. Add 2 cc. of nitric acid and boil on a hot plate to convert the pyro- to ortho-phosphate.

3. Continue boiling until the solution has evaporated to a volume of about 150 cc.

4. Cool and add 10 cc. of ammonium chloride solution (25 per cent) and 5 cc. of magnesium mixture.

5. Carefully add ammonium hydroxide until a precipitate forms, then dissolve the precipitate with a few drops of hydrochloric acid.

6. Dilute to about 200 cc. volume and cool.

7. Run in dilute ammonium hydroxide, drop by drop, with constant stirring, until the precipitation of the magnesium ammonium phosphate appears complete.

8. Add 60 cc. of strong ammonium hydroxide, stir, and allow to stand cold for at least 1½ hours.

9. Filter through a tared Gooch crucible, washing the precipitate with dilute ammonium hydroxide (1 + 10) and giving one final washing with ammonium nitrate solution (20 per cent).

10. Dry the precipitate on the hot plate, or in an oven, and ignite over a low burner, gradually increasing the size of the flame.

11. Ignite to constant weight over the full flame of a Bunsen burner.

12. Cool in a desiccator and weigh as magnesium pyrophosphate.

$$\text{Grams of Mg}_2\text{P}_2\text{O}_7 \times 1.1947 \times 100 = \text{percentage of Na}_2\text{P}_2\text{O}_7.$$

*(C) Estimation of orthophosphate:*

1. To the filtrate in (A) 8 add 20 cc. of hydrochloric acid and 2 or 3 cc. of nitric acid and evaporate on the hot plate to small volume (about 100 cc.) to remove the acetates.

2. Cool. Add ammonium hydroxide until a precipitate forms, then dissolve the precipitate with a few drops of hydrochloric acid.

3. Cool again, if necessary, and add dilute ammonium hydroxide (1 + 5) drop by drop, with constant stirring, until the precipitation of the magnesium ammonium phosphate appears complete.

4. Add 60 cc. of strong ammonium hydroxide, stir, and allow to stand cold for at least 1½ hours.

5. Filter and wash with dilute ammonium hydroxide (1 + 10).

6. Dissolve the precipitate back into the same beaker in which the precipitation was made by washing with dilute hydrochloric acid (1 + 10) and finally with water. The volume of the solution should be about 200 cc.

7. Add 5 cc. of magnesia mixture and then ammonium hydroxide until a precipitate forms. Dissolve the precipitate with a few drops of hydrochloric acid.

8. Run in dilute ammonium hydroxide (1 + 5) drop by drop with constant stirring, until the precipitation of magnesium ammonium phosphate appears complete.

9. Add 60 cc. of strong ammonium hydroxide and allow to stand for at least 1½ hours.

10. Filter through a tared Gooch crucible, wash, ignite, and weigh as in the determination of the pyrophosphate (B) 9 – 12.

$$\text{Grams of Mg}_2\text{P}_2\text{O}_7 \times 1.0781 \times 100 = \text{percentage of NaH}_2\text{PO}_4.$$

## REAGENTS.

*Magnesium acetate.*—Weigh out 100 grams of magnesium carbonate and 400 grams of ammonium acetate into a 1 liter casserole and add sufficient water to bring the volume

to about 800 cc. Carefully add 80 cc. of glacial acetic acid. Heat over a burner, gently at first and finally boiling for a few minutes to drive off the carbon dioxide. Filter and cool. Add glacial acetic acid, a few cc. at a time, until the solution is distinctly acid to methyl red indicator. Continue to add the acetic acid until the color becomes distinctly red (not orange). This will take 75–100 cc. more of the acid. Dilute to 1 liter.

*Magnesia mixture.*—Dissolve 55 grams of magnesium chloride ( $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ) in a small quantity of water. Add 140 grams of ammonium chloride and 350 cc. of ammonium hydroxide (sp. gr. 0.90). Filter and dilute to 1 liter.

NOTE: A sufficiently large sample should be taken to contain 0.25–0.50 gram of  $\text{Na}_4\text{P}_2\text{O}_7$  for each determination.

*Methods Submitted by W. C. Geagley, Lansing, Mich.*

1. *Molybdate method.*—Determine total phosphorus pentoxide after converting all  $\text{P}_2\text{O}_5$  into the ortho form, as in the usual method for fertilizers, digesting the sample in concentrated nitric acid<sup>1</sup>. Next determine  $\text{P}_2\text{O}_5$  in the ortho form by digesting the sample in water only, without the addition of strong acid, and in the molybdate precipitation use as little nitric acid and as low a temperature as possible, to avoid converting any pyrophosphate into orthophosphate.

2. *Berthelot and André Method*<sup>2</sup>.—To the very slightly acid solution, add a magnesia mixture made up of magnesium chloride, ammonium chloride, and ammonium acetate (5 grams of each in 100 cc. of water) sufficient to precipitate all  $\text{P}_2\text{O}_5$ . In case the orthophosphate precipitates at this point, dissolve by adding the least possible quantity of dilute acetic acid. Digest on the water bath for 3 hours; cool by standing; filter off pyrophosphate, washing with water slightly acidified with acetic acid; dry; ignite; and weigh as  $\text{Mg}_2\text{P}_2\text{O}_7$ . To the filtrate add one-third its volume of strong ammonia, stand over-night, filter off orthophosphate, wash with dilute ammonia, ignite to  $\text{Mg}_2\text{P}_2\text{O}_7$ , and weigh.

3. *Cadmium method.*—Separation as cadmium salts. Dissolve by standing in water with occasional shaking; add glacial acetic acid; and repeat, finally make up to volume (e. g., 500 cc. containing 75 cc. of acetic acid); and allow to stand overnight. To an aliquot add a large excess of concentrated cadmium chloride solution; agitate till a precipitate is produced, warming slightly if necessary. Let stand overnight. Filter off pyrophosphate, washing with dilute acetic acid. Oxidize, filter, and precipitate by evaporation to dryness with nitric acid twice. Also evaporate filtrate to dryness and oxidize with nitric acid. Determine  $\text{P}_2\text{O}_5$  in each portion by the usual magnesium pyrophosphate method.

The referee used a method essentially the same as that submitted by A. H. Fiske.

The collaborative results are shown in Table 4.

The collaborative results are not in close agreement, but they approximate each other, and it is believed that more work along this line will show great improvement in results.

The mixture sent to the collaborators was made up of equal weights of monobasic sodium phosphate ( $\text{NaH}_2\text{PO}_4 + \text{H}_2\text{O}$ ) and sodium pyrophosphate ( $\text{Na}_4\text{P}_2\text{O}_7$ ).

Since commercial sodium pyrophosphate may contain, also, sodium

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 2.

<sup>2</sup> *Z. anal. Chem.*, 1921, 60: 385.

TABLE 4.  
*Ortho- and pyro-phosphates.*

ANALYST	P <sub>2</sub> O <sub>5</sub> IN ORTHO	P <sub>2</sub> O <sub>5</sub> IN PYRO	TOTAL P <sub>2</sub> O <sub>5</sub>
A. H. Fiske	25.22	25.19	50.41
	25.18	25.13	50.31
W. E. Stokes	22.84	27.63	50.47
	22.92	27.55	50.47
W. C. Geagley	24.4 (Method 1)	22.6	47.00
	23.40 (Method 2)	23.95	47.35
	24.20	24.35	48.55
	25.30 (Method 3)	24.44	49.74
L. H. Bailey	26.25	25.32	51.57
	26.21	25.36	51.57

metaphosphate, it is desirable that this study be broadened so as to include the separation and estimation of meta-, pyro-, and ortho-phosphates in the presence of each other.

The referee gratefully acknowledges the assistance given by the Victor Chemical Works, Chicago Heights, Ill., and the Calumet Baking Powder Co., Chicago, Ill., in furnishing the samples for collaborative work, and wishes to thank the various collaborators for their splendid cooperation and their helpful suggestions and criticisms.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the present tentative method for the electrolytic determination of lead be made official.

(2) That the present tentative gasometric method for determining carbon dioxide be further studied, the results obtained by using a factor weight as given in the method being compared with those obtained by using a fixed weight and the temperature and pressure readings being taken at the time the volume of gas is read.

(3) That suitable methods be developed for the separation and estimation of meta-, pyro-, and ortho-phosphates in the presence of each other.

#### REPORT ON FLUORIDES IN BAKING POWDER.

By JAMES K. MORTON (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

The volatilization method for the determination of fluorine in baking powder as presented before this association in 1923<sup>2</sup> has been subjected

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 81.

<sup>2</sup> *This Journal*, 1924, 8: 101.

to further collaborative study. Acting on the suggestion of one of the collaborators of last year it was requested that the determination be made on a 10 gram sample instead of a 20 gram sample as directed in the method.

Slight changes in manipulation also have been suggested in this year's reports; they are not of sufficient importance to call for a revision in the original transcript, but they may be of interest to the analyst. F. L. Thayer substituted a plain U-tube containing glass wool for the straight tube specified for absorption. G. A. McDonald substituted a U-tube filled with wet glass beads for the test tube containing water and believes he secures a better absorption of the gas. One of the collaborators stated that this method was being used in his laboratory as a routine method with very satisfactory results.

M. H. Kemp called attention to an inaccuracy in the method that requires correction. He observed that chlorides were present in the final solution after titration with alkali and commented as follows: "When magnesium nitrate is used to assist in the ashing of the material some nitrate remains after the oxidation is complete. Digestion of this ash with anhydrous sulfuric acid yields some free nitric acid which causes the liberation of chlorine from chlorides present in the baking powder. This free chlorine is not absorbed in the silver sulfate solution but passes over with the silicon tetrafluoride into the water solution and is converted to hydrochloric acid yielding an increased acidity". Determinations were made by the associate referee on the A. O. A. C. sample with and without the use of magnesium nitrate, and chlorides were found in both instances but to a larger degree when nitrate was present. No suggestion was made for eliminating this possibility. It will be necessary to determine chlorides as well as sulfates in the solution after titration and correct the result accordingly.

The baking powder for the collaborative work reported was prepared through the courtesy of T. J. Bryan of the Calumet Baking Powder Company, to whom thanks are due. To the regular stock baking powder 0.2 per cent of fluorine was added as sodium fluoride. Determinations by Kemp on the original baking powder indicated that it contained 0.0105 per cent of fluorine. The prepared baking powder sample, therefore, contains 0.2105 per cent of fluorine.

#### COMMENTS ON RESULTS.

The results submitted by the collaborators are very good. On results uncorrected for chlorides six of the eight analysts are within a range of 0.009 per cent, and if all had corrected for chlorides it is believed this difference would be much less. All determinations were made on 10

*Collaborative data on the determination of fluorine in baking powder\*.*

ANALYST	FLUORINE FOUND		CORRECTED FOR SULFATE AND CHLORIDE	AVERAGE	RECOVERY
	Corrected for Sulfates	Average			
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Fred L. Thayer Rumford Chemical Co. Providence, R. I.	0.2033 0.2014 0.1900 0.2090	0.2005			95.4
A. H. Allen Virginia-Carolina Co. Richmond, Va.	0.178 0.173	0.175			83.1
H. L. Moxon Virginia-Carolina Co. Richmond, Va.	0.163				77.4
G. A. McDonald Victor Chemical Co. Chicago, Ill.	0.200 0.194 0.203	0.199			94.5
T. J. Scott Federal Phosphorus Co. Anniston, Ala.	0.194 0.196 0.203	0.197			93.5
Milton H. Kemp Calumet Baking Powder Co. Chicago, Ill.	0.2046 0.2070	0.2058	0.190 0.191	0.1905	90.5
James K. Morton	0.1938 0.1919 0.2052 0.1947	0.1984	0.1919 0.1881 0.1938 0.1919 0.1919 0.1919	0.1915	91.0
Percy O'Meara State Department of Agriculture Lansing, Mich.	0.203 0.209 0.197 0.200	0.2022			

\* Calculated to contain 0.2105 per cent.

gram samples. This may have contributed to more uniform results, but it is believed that added experience with this method was the deciding factor.

The method as amended is capable of producing very excellent results in the hands of a careful analyst. The factor of recovery remains about 90 per cent, and it is not probable that this will be greatly changed. Further collaborative work may bring more evidence to bear on this point, but it would still be necessary to use a factor and the method in this form may not be acceptable as an official method. It should be retained, however, as a tentative method for fluorine.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the volatilization method for the determination of fluorine in baking powder<sup>2</sup> be amended as follows: Delete the last three sentences and substitute the following: "After titration make the neutral solution to a definite volume and divide into two equal parts. Determine sulfates in one portion as directed on page 45, par. 17, and chlorides in the other as directed on page 87, par. 21. Calculate the results to the whole sample in terms of 0.1 *N* alkali and correct accordingly".

Weight of  $\text{BaSO}_4 \times 82.75 = \text{cc. of } 0.1 \text{ } N \text{ alkali.}$

Cc. of  $\text{AgNO}_3 \times 0.282 = \text{cc. of } 0.1 \text{ } N \text{ alkali.}$

Multiply the corrected result by the factor 1.1 to obtain the fluorine content.

(2) That no further collaborative work be undertaken at this time.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 82.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 314, par. 38; *This Journal*, 1926, 9: 45.

## DRUG SECTION.

Before the formal reports were given in the Drug Section, Dr. A. G. Dumez, who represented the United States at the second Conférence Internationale pour l'Unification de la Formule des Médicaments Héroïques, gave a brief review of the work accomplished at the meeting held in Brussels, Belgium, last year.

### REPORT ON DRUGS.

By ARTHUR E. PAUL (U. S. Food and Drug Inspection Station, Transportation Bldg., Chicago, Ill.), *Referee*.

It has been the experience of the Referee on Drugs that associate referees take much interest in their respective subjects and that they do splendid and thorough work. Nevertheless, experience has also shown that such intensive interest may not be expected in the matter of conformance to the regular procedure of this association, nor, in connection with specific methods, to other methods that have already been adopted and that are more or less connected with the methods under consideration. It was believed, therefore, that since the new edition of *Methods of Analysis* has just been completed, and also the 10th revision of the United States Pharmacopeia, it would be incumbent upon the referee to review carefully the reports submitted, with unification of the above features in view. For this reason all associates were urged to commence work on their respective subjects at the earliest possible time, and to submit their finished reports, if at all possible, by July 1st. It is a matter of much pleasure that in a considerable number of instances the reports were received at or near that date. On the other hand, it is deeply regretted that several associate referees found it impossible to submit any reports. This was due in each instance to interfering regular duties.

In harmony with this plan, the referee has made such comments or suggestions as appeared to him desirable in connection with the various topics which have been accepted for study. It has not been the intention, in any case, to add anything to the reports, but, as stated above, to harmonize them with the usual association procedure and with other methods that have already been adopted.

It is felt that excellent work has been done by the associate referees during this year and that the reports submitted are of much interest and value in advancing the status of the drug methods of this association.

The following comments, suggestions, and recommendations are submitted in connection with the topics under consideration:

## ACETYSALICYLIC ACID.

The recommendations submitted by the Associate Referee on Acetylsalicylic Acid are quite desirable, but they may require some modification in order to bring them in harmony with the usual procedure.

Recommendation 1—that the double titration method, now tentative, be made official—is somewhat questionable, since the method of extraction has been modified by the associate referee. Recommendation 5—that with the filing of this report this topic be considered closed—does not seem possible, so far as the titration methods are concerned.

For the sake of convenience, the complete amended recommendations are stated below:

(1) That the following tentative methods be made official:

- (a) Melting point, tentative (1922).
- (b) Free salicylic acid, qualitative, (1921).
- (c) Free salicylic acid, quantitative, (1921).
- (d) Total salicylates, bromine method, (1921).
- (e) Acetylsalicylic acid in mixtures (1923).
- (f) Combined acetic acid, two methods, (1924).

(NOTE: Relative to 1-e, attention is called to an error in the proceedings of 1923, since this method was at that time recommended by the associate referee for tentative adoption. Through an oversight, Committee B recommended official adoption and this was approved by the association. This action, it would seem, should not at this time prevent the method from being made official in accord with the recommendation of the present associate referee.)

(2) That the present tentative double titration method be amended and readopted in this form as tentative. The method, as revised by the referee, has been accepted and published by the Committee on Changes in Methods of Analysis<sup>1</sup> with the exception of the determination for the single titration method, which should read as follows: "Proceed with the dry chloroform extract as directed under 'Assay', U. S. P. X, p. 14".

(3) That next year's referee and associate referee give consideration to these single and double titration methods, looking to their final adoption by the association.

## ALCOHOL IN DRUGS.

The Associate Referee on Alcohol in Drugs tried the details which he worked out and obtained some remarkably close results, but he was not able, during the present year, to submit the method to collaborators for study. As described for the actual determination, the method differs somewhat as to details from the general method for alcohol, which is now official, but it is not believed that there is any actual necessity for any difference in the details for the final method of determination, whether the product examined is a food or a drug. It is believed that

<sup>1</sup> *This Journal*, 1920, 9: 49.



if the details advocated by Lynn are preferable to those which are now official they should be referred for consideration to the Referee on Determination of Specific Gravity and Alcohol.

The associate referee submitted no recommendation, but it is suggested that his details, slightly amended as explained above, be subjected next year to careful collaborative study on samples of known composition, with adoption in view as a tentative method. The following form is suggested for consideration by next year's associate referee:

#### PREPARATION OF SAMPLE.

Use a sample containing not more than 20 grams of absolute alcohol. Unless the alcoholic strength is too great, a 100 cc. sample is satisfactory.

If a large quantity of solids is present, dilute sufficiently with water, distil off 150 cc., and proceed as directed in 3 and 4<sup>1</sup>, or use one of the following methods:

If *iodine* is present, add an excess of zinc dust, and when reduction is complete, proceed as directed in 3 and 4.

If *glycerin* is present, use no special precautions unless the residue in the flask after distillation contains in excess of 50 per cent of this constituent. If this is the case, treat the distillate as an original sample.

If a *volatile acid* is present, neutralize with 10 per cent sodium hydroxide solution or an excess of magnesium oxide. If *phenol* is present, add an excess of 10 per cent sodium hydroxide. If a *volatile base* is present, neutralize with dilute sulfuric acid (1 + 9) or phosphoric acid (1 + 9).

If *camphor*, *ether*, *chloroform*, *amyl nitrile*, *benzaldehyde*, or a *volatile oil* is present, dilute, if necessary, so that not more than 15 per cent of alcohol is present. Saturate with sodium chloride and shake the sample with about 20 cc. of petroleum ether. Separate and re-extract the aqueous layer with petroleum ether. Wash the combined petroleum ether with 5 cc. of saturated salt solution. Determine alcohol in the aqueous layers. (If the extractives make the shaking-out process difficult, the extraction with petroleum ether may be carried out after distillation.)

If *iodoform* is present, add 5 cc. of washed chloroform and proceed as directed previously for chloroform.

If *chloral hydrate* is present, add an excess of saturated sodium hydroxide solution in a stoppered flask. Allow to stand 30 minutes and proceed as directed for chloroform.

If *formaldehyde* is present, add 50 cc. of hydrogen peroxide (U. S. P.) and an excess of saturated sodium hydroxide solution and heat on a water bath under a reflux condenser until effervescence ceases. Proceed as directed under 3 and 4.

If *acetone* is present, add an excess of benzaldehyde (usually 5 cc. will suffice) and 10 cc. of 20 per cent sodium hydroxide solution, and heat on a water bath under a reflux condenser for 30 minutes. Cool, and treat as directed for benzaldehyde.

#### DETERMINATION.

If necessary, dilute the sample containing not in excess of 20 grams of alcohol directly, or preferably by one of the methods described previously, to 150 cc. Distil and determine the alcohol as directed in 3 and 4.

In connection with the work on alcohol, it would seem that some attention should be given to the procedure to be used for the determination of alcohol when the proportion present is very small. This was made

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 361.

a special topic in 1922, but no work was performed at that time<sup>1</sup>. A suitable statement should be included under "Preparation of Sample" to take care of such instances.

#### ARSENICALS.

It is noted that the U. S. Pharmacopeia includes an assay for arsenicals, which, however, is in effect the titration of the sodium in the sample examined. The method proposed by the associate referee has the advantage that it is a determination of the active constituent arsenic. It is considered desirable, therefore, that his method be approved by this association.

#### CAMPHOR.

The recommendation of the Associate Referee on Camphor, that the method proposed by him be tentatively adopted, appears fully warranted in view of the satisfactory collaborative results which he has reported. For the sake of simplicity, however, a slight change in the method of calculation and slight changes in the wording of the method are suggested. The method, including these changes, has been published.<sup>2</sup>

#### MONOBROMATED CAMPHOR.

The Associate Referee on Monobromated Camphor made no recommendation, but in a previous letter addressed to the referee he stated: "As to camphor monobromated, I think the method published on p. 587 of Volume 5 of the *Journal* is entirely satisfactory and should be made official".

Undoubtedly, the associate referee intended to recommend that the present tentative method<sup>3</sup> be made official, and such action is now recommended. Relative to the additional tentative method, No. 2, p. 394, no opinion was expressed by the associate referee. At the 1921 meeting study of the method, with simplification in view, was suggested. However, since method No. 1 is preferred, it would seem undesirable to suggest any change in the status of method No. 2.

There appears no urgent need to continue these topics except to recommend, in due course, that the tentative method for camphor, if it be so adopted in this meeting, be made official.

#### CHAULMOOGRA OIL.

The work of the Associate Referee on Chaulmoogra Oil during the last two years has been essentially in the nature of a scientific investigation, and it is considered that a valuable contribution has been made to the literature on the subject.

<sup>1</sup> *This Journal*, 1924, 7: 133.

<sup>2</sup> *Ibid.*, 1926, 9: 52.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 393.

Since last year's report, the 10th revision of the U. S. Pharmacopeia has been completed, and among the newly included items is chaulmoogra oil. To what extent the tests given for this product were influenced by the work of this associate referee is not known, but it is noted that essentially the U. S. P. tests, together with additional ones, were studied by him. It seems unnecessary to present these tests for tentative adoption, since the Pharmacopeia is specifically recognized in the food and drugs act. The associate referee also recommends that certain other methods be adopted as tentative. These methods have been given some consideration, and for the purpose of conformity with other A. O. A. C. methods they have been slightly amended by the referee.

The methods that are not included in the U. S. Pharmacopeia but recommended at this time for tentative adoption have been published<sup>1</sup>.

It is recommended that a study be made of any available color reactions, or other specific tests for chaulmoogra oil, particularly the color identity test described by Lifschutz<sup>2</sup>.

It is also suggested that consideration be given to loss or gain in heating, which is mentioned by the associate referee in his conclusion.

#### CHLORAMINE PRODUCTS.

As the recommendations of the Associate Referee on Chloramine Products appear to be desirable, the suggestion that this subject be referred to the Referee on Food Preservatives is approved.

#### CHLOROFORM AND CARBON TETRACHLORIDE.

The work of the Associate Referee on Chloroform and Carbon Tetrachloride shows that he has made much progress in the development of a suitable method for the accurate determination of these two substances. Several collaborators submitted results which apparently are quite satisfactory, while some of the reported results deviate rather widely from those obtained by the associate referee.

It is regretted that the samples examined by the collaborators were prepared from commercial products, the purity of which was relatively unknown. Doubtless the associate referee considered that for this essentially preliminary work variations would be encountered in excess of the extreme discrepancies which might be due to impurities in the samples. The associate referee mentions certain additional features which should be taken into consideration in carrying on further work on this subject.

It is believed that with the experience recorded in the present associate referee's report, it will be possible to obtain some very satisfactory results next year, with a view to the adoption of the methods as tentative.

<sup>1</sup> *This Journal*, 1926, 9: 52.

<sup>2</sup> *Chem. Ztg.*, 1921, 45: 1264.

Further study of the associate referee's method, with samples of substances as nearly pure as can be obtained or prepared, is now recommended.

#### IPECAC ALKALOIDS.

The condition relating to the subject of ipecac alkaloids is rather peculiar. The method of preparation of the fluidextract of ipecac, as given in U. S. Pharmacopeia X, is somewhat different from that given in U. S. P. IX, and the assay standard has been changed from a minimum of 1.8 grams per 100 cc. to a minimum of 1.35 grams. The assay method has also been entirely changed. The new method, it seems, is a slight modification of the U. S. P. method for fluidextract of belladonna root.

During the preparation of the new U. S. Pharmacopeia, Palkin and Watkins published<sup>1</sup> the results of an investigation on the determination of alkaloids in nux vomica preparations, which involves the removal of resins by the use of acid prior to extraction. Subsequently Palkin, Murray, and Watkins<sup>2</sup> recorded results obtained by this procedure on various fluidextracts, including ipecac. In this investigation the authors used a mechanical extractor which they devised. It facilitated the extraction operation and yielded practically the same results.

While the U. S. P. X method is now official for all regulatory work, it seems probable that the methods of these authors may include features that will permit more expeditious examinations, and possibly yield more accurate results. It would seem, therefore, that a further study of their methods would be desirable. It is recommended that collaborative study be devoted to these procedures and that the method be compared with the U. S. P. X method. Since the methods are somewhat scattered and have not been repeated by the associate referee they are given here in full:

#### FLUIDEXTRACT OF IPECAC.

##### PREPARATION OF SAMPLE.

Pipet 20 cc. of the sample into a small beaker. Add 5 cc. of normal sulfuric acid and evaporate on a steam bath with the aid of an air blast to a volume of about 10 cc. or less. Transfer the entire alkaloidal solution to a 100 cc. volumetric flask and add about 30 cc. of water while rotating the flask; cool, and dilute to volume. Allow to stand 5 minutes and filter.

##### *Method No. 1—Hand Extraction.*

Pipet 20 cc. of the filtrate (representing 4 cc. of the original sample) into a separatory funnel. Add 2 cc. of dilute ammonia solution (8 per cent ammonium hydroxide) and extract the alkaloids with equal volumes of ether at least eight times, or until complete. Test the final extracted residue with Mayer's reagent.

<sup>1</sup> *J. Am. Pharm. Assoc.*, 1924, 13: 691.

<sup>2</sup> *Ind. Eng. Chem.*, 1925, 17: 612.

Transfer the ether extract to a second separatory funnel, wash with 10 cc. of water, and withdraw the ether to a beaker or 200 cc. Erlenmeyer flask. Evaporate the combined extracts on the steam bath, using an air blast. Warm the alkaloidal residue with 2-3 cc. of neutral alcohol on the steam bath to insure complete solution. Add 10 cc. of 0.1 *N* sulfuric acid, or equivalent; dilute with about 20 cc. of water; and titrate the excess acid with 0.02 *N* alkali, using methyl red indicator. One cc. of 0.1 *N* acid = 24 mg. of ether-soluble alkaloids of ipecac.

*Method No. 2—Mechanical Extraction.*

Pipet 20 cc. of the filtrate (representing 4 cc. of the original sample) into a mechanical extractor which has been fitted to a 200 cc. Erlenmeyer flask. (The form described by Palkin, Murray, and Watkins is very satisfactory.) Add 2 cc. of dilute ammonia solution (8 per cent ammonium hydroxide) and about 25 cc. of ether. Shake gently to prevent the settling of any solid matter on the bottom of the extractor, then add ether until about 50 cc. overflows into the flask. Heat the flask on a steam bath and extract for 2 hours, or until complete. Separate the ether from the aqueous layer and add it to the main concentrate in the flask. Evaporate the combined ether extract on a steam bath, add 2-3 cc. of absolute alcohol, and repeat the evaporation to remove all traces of ammonia. Titrate the alkaloids as in Method No. 1.

With reference to the recommendation made by the associate referee, that a gravimetric modification be further studied, it may be stated that the experience generally throughout the Bureau of Chemistry laboratories, so far as the referee is advised, has been that volumetric methods, where applicable, are preferable to gravimetric methods in the determination of alkaloids. It hardly seems desirable, therefore, to continue the study of the gravimetric details.

RADIO ACTIVITY IN DRUGS AND WATER.

Early in the year the Associate Referee on Radio Activity in Drugs and Water submitted plans for this year's investigation which entailed a considerable amount of work. Later he reported that a part of this work had been done, but that additional data must be secured before a satisfactory report could be prepared. Under the circumstances, it is respectfully recommended that this topic be continued next year.

LAXATIVES AND BITTER TONICS.

It is regretted that the Associate Referee on Laxatives and Bitter Tonics was unable to carry on any collaborative work. Unforeseen circumstances interfered with his plans. It is understood, however, that he has conducted some research on the subject. This work should be continued next year.

MERCURIALS.

Last year the Associate Referee on Mercurials investigated two methods for the determination of mercury in antiseptic tablets contain-

ing mercuric chloride. These were the iodide method of G. S. Jamieson<sup>1</sup> and the formaldehyde method of E. Rupp<sup>2</sup>. The report submitted by him last year indicates no preference for either method, so far as anti-septic tablets are concerned.

This year he devoted his attention to tablet triturates of mercuric chloride and found that the Jamieson method was not applicable to this class of substances, but that a modification of the Rupp method, in which the milk sugar present was utilized as reducing agent in place of added formaldehyde, gave promising results.

It is noted that the new revision of the U. S. Pharmacopeia includes the determination of mercury in a number of mercury compounds, including mercuric chloride. Under these circumstances, the topic might be deemed closed. Nevertheless, there is a doubt as to the applicability of the U. S. P. methods to tablet triturates of mercurous or mercuric iodide or chloride owing to the presence of milk sugar.

It is respectfully recommended that during the coming year the method proposed by the associate referee, as well as the official U. S. P. methods, be subjected to collaborative study, particularly in connection with interfering substances which are liable to be encountered.

#### PYRAMIDON.

The present status of the work on pyramidon is decidedly complicated. An unusually large number of methods have been proposed, and all of them seem quite sound. The history in brief may well be stated at this time.

In 1922 Associate Referee Hanson submitted the extraction, precipitation, and titration methods<sup>3</sup>.

These methods were studied by Hanson, but they were not submitted to collaborators, and he recommended that the first two of these be further studied. He, manifestly, discarded the third method. This recommendation was approved by the association<sup>4</sup>.

In 1923 Hanson proposed, studied, and recommended for tentative adoption, four qualitative tests, which were approved by the association. He also further studied the two quantitative methods, but again recommended them for further study<sup>5</sup>. This action was approved by the association<sup>6</sup>.

In 1924 Hanson recommended that the four qualitative methods be made official, and the first action was taken by the association. He again submitted his two quantitative methods to collaborative study, but deemed it desirable to include certain minor details. This was not done

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<sup>1</sup> *This Journal*, 1925, 8: 538.

<sup>2</sup> *Ibid.*, 539.

<sup>3</sup> *Ibid.*, 1923, 7: 31.

<sup>4</sup> *Ibid.*, 6: 271.

<sup>5</sup> *Ibid.*, 1924, 8: 40.

<sup>6</sup> *Ibid.*, 7: 275.

in the methods as submitted to collaborators. He finally recommended that the methods be still further studied for another year. However, Committee B recommended tentative adoption of these methods as re-written by the associate referee, and this action was approved by the association<sup>1</sup>.

For the present year, however, Associate Referee Rabak proposed further slight modifications of the now tentative extraction method, which modifications appear to be advantageous. The method as re-written by Rabak was not submitted to collaborators, but it was studied by him and yielded favorable results.

It is believed, since neither of the two tentative methods nor Rabak's modifications have been studied collaboratively, that the now tentative hydrochloric acid method and the extraction method, as amended by the associate referee, should be made official next year, but that both should be submitted to collaborators during the coming year in order that this topic may be closed.

#### QUININE AND STRYCHNINE—SEPARATION.

The results reported by the Associate Referee on Quinine and Strychnine are as satisfactory as may be expected in view of the difficulty of this separation. His recommendation that the method be adopted tentatively, therefore, seems to be desirable.

#### SILVER PROTEINATES.

While there seems to exist some doubt as to the relation between the ionic silver content of silver proteinates and their clinical activity, the prevailing opinion in the medical profession is that there does exist such relation. Under the circumstances, the determination of the ionic silver is of considerable importance. The tentative dialysis method, devised by Eaton<sup>2</sup>, yields very satisfactory results, but it is rather time-consuming. The method now proposed by the associate referee is quicker of application and, in his opinion, has the further advantage that it measures the antiseptic power, which is the important property of the compound.

In view of the importance which is at this time attached to this class of compounds, it is considered desirable to study the new method in conjunction with the present tentative method, as suggested by the associate referee, as well as to approve the recommendations made by the associate referee.

#### NITROGLYCERIN.

The associate referee has studied and submitted to collaborators two methods for the determination of nitroglycerin, both of which, however,

<sup>1</sup> *This Journal*, 1925, 8: 268.

<sup>2</sup> *Ibid.*, 551.

are modifications of a procedure devised by Devarda. The first of these modifications was prepared by A. G. Murray, and the second by the associate referee, A. W. Hanson. Both modifications seem to yield satisfactory results, but the details of Hanson's method seem to possess certain advantages, particularly in the way of simplicity.

Hanson's method, it will be noted, involves extraction with ether, spontaneous evaporation in the presence of alcohol, and transfer to the reduction flask. The associate referee also attempted direct reduction of the tablets in the presence of the vehicle and excipient. This yielded unsatisfactory results. He then placed the tablets into a volumetric flask, diluted with alcohol to volume, and transferred an aliquot directly to the reduction flask. Owing to the volume occupied by the milk sugar, the results were high. Calculation will show that the extent of this discrepancy corresponds approximately with the theoretical expectancy. Hanson also extracted the material with alcohol, filtered, diluted to volume, and transferred an aliquot to the reduction flask. These results were satisfactory.

It is believed that a further experiment would be highly desirable, namely, to add to the tablets in a small glass-stoppered flask an accurately measured volume of absolute alcohol, crush the tablets with a stirring rod, shake, allow to settle, and withdraw an aliquot. This procedure, it is believed, will correspond in accuracy to the new method studied this year and will result in materially shortening the procedure. It is suggested that a number of tablets, corresponding to 1 grain of nitroglycerin and 50 cc. of alcohol, will probably be satisfactory, 25 cc. of the solution then conforming to the quantity used in Hanson's details.

It is believed that it would be desirable to study this further slight modification next year, with tentative adoption of the entire procedure in view. It is also believed that with the adoption of the Devarda method, one or both of the present colorimetric methods may be discontinued.

#### APOMORPHINE.

The fairly satisfactory results obtained by the five experienced drug men who worked on apomorphine would indicate that the method is sound. However, the associate referee recommends further study and proposes that certain features be given attention during the coming year. It is believed that these recommendations are desirable.

#### SANTONIN.

The Associate Referee on Santonin submitted no report prior to the meeting. He stated, however, that he had done considerable experimental work which will be valuable to next year's associate referee as a possible basis for collaborative work.



## ETHER.

Ether, which is required to be declared if present in drugs, presents no little difficulty in its identification and determination. It has been considered desirable to carry on an investigation as to satisfactory methods, but in view of the difficulty of the problem and the relatively infrequent use of ether in medicines for internal or external application, and further, in view of the fact that a number of important topics claim immediate attention, no appointment of an associate referee was made this year. It is left for the next Referee on Drugs to decide whether or not such appointment should be recommended for the coming year.

## BIO-ASSAY OF DRUGS.

No copy of the report of the Associate Referee on the Bio-assay of Drugs, E. W. Schwartze, was received. He has, however, done some work on the assay of thyroid preparations. It is the understanding that some of these methods are still quite new, and more or less in process of development.]

## BARBITAL AND PHENOBARBITAL (VERONAL AND LUMINAL).

Barbital and phenobarbital were studied for two years, and a quantitative method applicable to the determination of either was adopted as a tentative method. Several qualitative tests were studied during the same period, but they were not recommended for adoption. In view of the fact that qualitative tests for both substances are included in U. S. Pharmacopeia X, it does not seem necessary for this association to investigate these tests further.

The U. S. P. methods for the determination of the melting point, as applied to these products, was also tentatively adopted, but it seems quite unnecessary for the association to retain them.

Under the circumstances given above, it is recommended (1) that the present tentative quantitative method, applicable to both barbital and phenobarbital, be adopted as an official method; (2) that the present tentative method for the determination of the melting point be dropped by this association; and (3) that these two topics be now considered closed.

## METHYLENE BLUE.

An iodometric assay for methylene blue was adopted tentatively in 1922, and subsequently a study was made of methods for determining moisture in this drug, but the new revision of the Pharmacopeia gives directions for this determination and thus makes further attention unnecessary.

It is now recommended that the present tentative method be adopted as an official method and that the topic be considered closed.

## PAPAIN.

The subject of papain was assigned last year, but no report was submitted by the associate referee. It was the consensus of opinion among those who were best qualified to judge that papain is used but little at this time and the product is considered of relatively little importance. It was considered the better policy to devote no attention to this topic, but to use the time on some of the more important unsolved problems.

## PHENOLPHTHALEIN.

The subject of phenolphthalein was practically closed with last year's report. The iodination method and the ether extraction methods were adopted as official methods (first reading). Final action should automatically follow this year.

The details for chocolate-containing products were adopted tentatively last year. Since they have been included in the new edition of *Methods of Analysis* under "preparation of sample" for the iodination method, it is assumed that these details will automatically become official when the two methods above mentioned are finally adopted.

## PHENYLCINCHONINIC ACID (ATOPHAN, CINCHOPHENE).

The subject of phenylcinchoninic acid was dropped because it has been included in U. S. Pharmacopeia X. It seems unnecessary to continue work on the subject, or to include the method in the A. O. A. C. reports.

## TURPENTINE.

Owing to its relation to pharmacy, the subject of turpentine has formerly been reported in the drug section, but it has been assigned to the Referee on Naval Stores.

## RECOMMENDATIONS FOR NEXT YEAR'S REFEREE.

It is recommended—

(1) That the following topics, which were studied this year, be discontinued:

Camphor and monobromated camphor,  
Chloramine-T products, and  
Separation of quinine and strychnine.

(2) That the following topics be continued:

Acetylsalicylic acid,  
Alcohol in drugs,  
Apomorphine,  
Arsenicals,  
Bioassay of drugs,

Chaulmoogra oil,  
Chloroform and carbon tetrachloride,  
Ether,  
Ipecac alkaloids,  
Laxatives and bitter tonics,  
Mercurials,  
Nitroglycerin,  
Pyramidon,  
Radio activity of drugs,  
Santonin, and  
Silver proteinates.

- (3) That the following new topics be assigned to associate referees:  
Cocaine,  
Crude drugs,  
Microchemical alkaloid methods, and  
Terpin hydrate.

#### REPORT ON ACETYLSALICYLIC ACID.

By CHANNING W. HARRISON (U. S. Food and Drug Inspection Station,  
Baltimore, Md.), *Associate Referee*.

The work undertaken involved: (1) A collaborative study of the titration method for the determination of acetylsalicylic acid and its comparison with a simple saponification method that specifies alcoholic potash as the saponifying reagent; (2) the relative efficacy of wet and dry extraction methods for removing acetylsalicylic acid from excipients.

For the purpose of making these studies two samples were sent to collaborators. One sample consisted of a commercially pure powdered acetylsalicylic acid showing a negative test for free salicylic acid, to be used in connection with the first study; the second sample consisted of powdered acetylsalicylic acid tablets containing excipients, to be used in the second study.

These samples, subdivided, were sent to six individuals whose names appeared on the list furnished the associate referee as those willing to collaborate on drugs. Results were subsequently received from three collaborators.

Accompanying the samples was a memorandum outlining the nature of the study to be made and requesting the collaborator to express his preference between the wet and dry procedure for the extraction of acetylsalicylic acid and also to make any other suggestions or criticisms of the methods.

The methods of analysis and instructions to collaborators were as follows:

*Sample No. 1.—Acetylsalicylic Acid—No Excipients Present.*

This sample is to be used in studying (a) the double titration method for the determination of acetylsalicylic acid, and (b) a simple saponification method with alcoholic potash.

For the double titration method follow the directions published previously<sup>1</sup>.

For the saponification method proceed as follows:

Weigh accurately 1 gram of sample and transfer to a 250 cc. Erlenmeyer flask. Pipet 25 cc. of approximately 0.5 *N* alcoholic potash solution into the flask. At the same time measure out a blank into another 250 cc. Erlenmeyer flask, using the same pipet and draining for the same length of time. Boil the contents of the flasks gently on the steam bath for about 15 minutes, placing a funnel in the neck of the flask to prevent excess evaporation of the alcohol. Cool the contents of the flask and titrate the sample and blank with standard 0.5 *N* acid, using phenolphthalein as indicator. Calculate the results by the following formula: Number of cc. of acid required for the blank — the number of cc. required by the sample  $\times 0.045015 \times 100$  = the percentage of acetylsalicylic acid.

*Sample No. 2.—Powdered Tablets of Acetylsalicylic Acid With Excipients Present.*

This sample is to be used in the determination of the relative merits of the dry and wet methods of extraction with chloroform.

*Dry method of extraction.*

Weigh accurately 1 gram of sample and transfer to a small beaker with 10 cc. of chloroform. Transfer the contents of the beaker to a dry filter paper and filter into a tared 250 cc. Erlenmeyer flask. Wash the beaker paper and funnel repeatedly with small portions of chloroform until the extraction is complete as shown by evaporating a small quantity of the chloroform on a watch glass. When the extraction is complete, place the Erlenmeyer flask containing the chloroform extractions on the steam bath. Evaporate off the chloroform, using suction to remove the vapors. Avoid exposing the contents of the flask to the full heat of the bath when the evaporation is nearing completion. After the chloroform is completely removed, dry the flask overnight in a vacuum desiccator and weigh accurately. In making the weighings, to compensate for moisture condensation, etc., use a counterpoise flask of the same size, similarly exposed. Calculate the percentage by weight of chloroform extract.

Determine acetylsalicylic acid in the chloroform extract, using the double titration method and report percentage by weight on the basis of the weight of sample taken.

*Wet extraction method.*

Weigh accurately 1 gram of sample and transfer to a small separatory funnel containing about 20 cc. of water. Shake out with repeated portions of chloroform, using 30, 25, 20, 10, 10, and 5 cc. portions, respectively, and testing by evaporation on a watch glass a portion of the final extraction to insure that the extraction is complete. Collect the chloroform fractions in a beaker and filter through a plug of absorbent

<sup>1</sup> *This Journal*, 1922, 5: 583, Method VI.

cotton into a tared 250 cc. Erlenmeyer flask, rinsing the funnel and cotton with chloroform. Evaporate off the chloroform and dry and weigh as directed in the dry extraction method.

Report the percentage by weight of chloroform extract and also acetylsalicylic acid by the double titration method on the basis of the original sample.

Results were received from the following collaborators: E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif.; C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.; R. W. Hale, U. S. Food and Drug Inspection Station, Baltimore, Md.

The results obtained by the collaborators and those of the associate referee are given in Tables 1 and 2.

TABLE 1.

*Collaborative results on determination of acetylsalicylic acid by double titration and by saponification.*

SAMPLE NO. 1	EATON†	GLYCART	HALE	HARRISON	
Double titration*.....	99.6 99.7	99.0 99.15	99.48 99.03	99.03 99.03	
				Average	99.25
Saponification*.....	99.0 99.0	99.00 98.88 98.33	99.26 99.48	99.03 99.48 99.26	
				Average	99.07

\* All results are expressed as percentage by weight of acetylsalicylic acid.

† "End point drags; not sharp." Double titration method.

TABLE 2.

*Collaborative results showing the efficiency of wet and dry methods of extracting acetylsalicylic acid with chloroform from excipients.*

SAMPLE NO. 2	EATON†	GLYCART‡	HALE	HARRISON§	
<i>Wet Extraction*</i>					
(a) Gravimetric.....	82.2	81.78 81.64	82.08 82.23	82.65 81.80	
				Average	82.05
(b) Titration .....		81.47 81.45	81.93 82.38	79.32 80.22	
				Average	81.13
<i>Dry Extraction*</i>					
(a) Gravimetric.....	81.0	81.36 81.20	82.07 82.30	82.75 82.25	
				Average	81.85
(b) Titration .....	79.6	81.40 80.77	82.38 82.60	80.13 79.32	
				Average	80.89

\* All results are expressed as percentage by weight of acetylsalicylic acid.

† "If lubricants are present, the weight of chloroform soluble would be of little value. Proper drying without loss would appear to be difficult."

‡ "The wet extraction method appears to separate the acetylsalicylic acid from the excipient more readily than the dry method and is therefore preferable. However, it was found that in the case of coated tablets this method failed to give results, whereas the dry method was suitable. It is my opinion that both methods are useful."

§ "Required a longer time and greater volume of solvent to complete the extraction of the acetylsalicylic acid when the dry method of extraction was used."

## DISCUSSION OF RESULTS.

The results given in Table 1 show that a simple saponification with alcoholic potash gave about the same results when determining acetylsalicylic acid as those obtained when the double titration method was used. Both methods are open to the general criticism that they are applicable only when the acetylsalicylic acid is uncontaminated with interfering substances such as free acid, easily saponified esters, or, in the case of the alcoholic potash saponification, any saponifiable oil.

Eaton calls attention to the rather unsatisfactory end point obtained in the double titration method. The associate referee has also frequently noted this fact and has therefore generally preferred the saponification method with alcoholic potash, because this difficulty is not encountered. Both of these methods are valuable as a check on the purity of a weighed residue supposed to be acetylsalicylic acid.

The results given in Table 2 show that on the average the recovery of acetylsalicylic acid from excipients was greater when the chloroform extraction was made from a liquid menstruum than when made from a dry extraction.

Eaton notes that if lubricants are present the weight of the chloroform extract is not a correct measure of the quantity of acetylsalicylic acid present. This undoubtedly is a fact; therefore, as a check on the purity of this residue it is always advisable to determine the acetylsalicylic acid present by the double titration or saponification method.

Glycart states that the wet extraction method separates the acetylsalicylic acid from excipients more readily than the dry method. This is in accord with the observations of the associate referee. He further states, in the case of coated tablets, that the dry method of extraction had given better results than the wet method and he therefore believes that both procedures should be retained, the choice being optional with the analyst. This is a good suggestion, and one with which the associate referee agrees, though he has always favored the wet method of extraction because it is quicker and requires less chloroform to complete.

RECOMMENDATIONS<sup>1</sup>.

With a view to closing this subject this year, it is recommended—

- (1) That the double titration method, now tentative, be made official.
- (2) That the saponification method with alcoholic potash, as given in this report, be made a tentative method.
- (3) That the dry method of extracting acetylsalicylic acid from excipients with chloroform, as given under the double titration method, be extended to include the optional wet extraction procedure as detailed in this report.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9, 76.

- (4) That the following tentative methods be made official:
- (a) Melting point (tentative 1922).
  - (b) Free salicylic acid, qualitative, (tentative 1921).
  - (c) Free salicylic acid, quantitative, (tentative 1921).
  - (d) Total salicylates, bromine method, (tentative 1921).
  - (e) Acetylsalicylic acid in mixtures (tentative 1923).
  - (f) Combined acetic acid, 2 methods, (tentative 1924).
- (5) That with the filing of this report this topic be considered closed.

## REPORT ON DETERMINATION OF ALCOHOL IN DRUG PRODUCTS.

By E. V. LYNN<sup>1</sup> (University of Washington, Seattle, Wash.), *Associate Referee*.

From an examination of the literature and of the method finally recommended in the previous communication<sup>2</sup>, it appeared that the distillation method for the determination of alcohol in drug products is reliable and sufficient.

It has been found that a 250 cc. round-bottom flask is most convenient and furnishes satisfactory results; a smaller flask occasionally results in foaming over. The flask should be connected by means of a spray trap to an upright condenser, preferably of the spiral type and of sufficient length; at least three feet of spiral tubing would appear to be the minimum length. The end of the condenser should be fitted with an adapter with the lower end extending at least one-half inch into the neck of a 50 cc. graduated flask used as a receiver. Previous work directed that the end of the adapter extend through the neck and into the bulb of the receiver and that cotton be packed loosely around the stem of the adapter to prevent air currents. It has been found that these precautions are unnecessary, although the use of cotton may be of some advantage.

A suitable volume of the preparation measured at 20°C. is introduced into the flask and diluted to 100 cc. with water. The quantity of alcohol should not be over 10 cc. The following experiments show the value of this precaution:

	I	II	III	IV	V	VI	VII
Volume of absolute alcohol in the							
100 cc. ....	12.88	11.06	9.95	5.56	2.95	1.72	0.72
Recovered in receiver. ....	12.66	10.93	9.93	5.54	2.90	1.71	0.70

The flask is heated in such a way that no charring results (it may be necessary to use a salt bath), and the distillation is carried on at a

<sup>1</sup> The associate referee was ably assisted in this work by Leonard Rhodes, University of Washington, Seattle, Wash. The report was presented by V. K. Chesnut.

<sup>2</sup> *This Journal*, 1922, 5: 537.

moderate rate. To obtain satisfactory results under the conditions of the work described in this report, 25 minutes was found necessary for the 50 cc. flask. Usually it is not necessary to immerse the receiver in ice water, but this procedure may be better in warm weather. When the flask is filled nearly to the mark the distillation is discontinued and the temperature adjusted to 20°C. It is then filled to the mark with water, mixed thoroughly, allowed to stand in the balance case for at least one hour, and weighed. It has been found that the full hour is necessary to get correct weights. A 50 cc. flask is practically as reliable as a pycnometer for making the gravity determination as repeated checks in the writer's laboratory have shown. The specific gravity is calculated, and the index of refraction is determined; from these results the percentage of alcohol in distillate and original is ascertained.

Tannin is fairly satisfactory to prevent frothing, but the writer has found that sulfuric acid equivalent to 5 cc. of the concentrated acid gives best results. The heating must be very gradual at first and then increased slowly to the usual distillation.

In one or two cases it has been found that the preparation must be diluted to more than 100 cc. because of a large quantity of contained extractives; in this event the preparation has been diluted greatly, distilled into 100 cc., and then redistilled as specified previously.

If the preparation contains *iodine*, zinc dust is added in sufficient quantity, and the distillation is begun only after reduction is complete. The careful use of sodium thiosulfate is also satisfactory, but it offers some difficulty. Zinc has been found efficient and accurate.

In the presence of a large quantity of *glycerin*, it has been found best to proceed as in the case of a large quantity of other extractives.

If the preparation contains a *volatile acid or base*, it has been found sufficient to neutralize it before distilling.

The presence of *volatile oils* or similar materials renders necessary an extraction before distillation. The writer has found that the method advocated by Murray<sup>1</sup> is perfectly satisfactory, except that the dilution should not be in excess of 15 per cent alcohol, and 20 cc. of petroleum ether should be used at each shaking out.

In the presence of *iodoform*, which is not probable, 5 cc. of chloroform may be added, and the same procedure used as for preparations containing chloroform.

In case *chloral hydrate* is present, the preparation is mixed with an excess of sodium hydroxide solution and allowed to stand one-half hour. Moraw suggested that the upper limit of alkali be designated, but the experience of the writer has been that a large excess of the latter, within

<sup>1</sup> *This Journal*, 1922, 5: 530.



reason, does no particular harm. Murray directed standing for ten minutes, but with fairly large quantities of chloral this is not sufficient time.

In the presence of *acetone* it is best to add an excess of both benzaldehyde and sodium hydroxide and heat on the water bath under a reflux for 30 minutes before proceeding as for benzaldehyde. The mercuric oxide method is not at all satisfactory, and mercuric sulfate is not much better. By the process suggested the acetone is completely removed and the alcohol unaffected.

In the presence of *formaldehyde* the U. S. P. assay method has been found to decompose the aldehyde without affecting the alcohol content.

The number of tests which have been suggested for the presence of *methyl alcohol* is legion, but it is considered that none of those commonly used is satisfactory. The method proposed by Murray<sup>1</sup> or the methods given in either U. S. P. VIII or U. S. P. IX are as good as any tried in this work. At present the writer is in search of a better method.

The presence of *vanillin*, *arsenic salts*, or *mercury salts* does not interfere with the usual distillation. It is possible that organic compounds of arsenic or mercury might sometimes vitiate the alcohol determination without some precautionary measure. The presence of *phosphorus* might interfere, but as it is insoluble in 10 per cent alcohol it could easily be removed if it were present, which is not probable.

*Phenol* acts as a volatile oil, but in large quantities it is not removed as in the ordinary procedure. Under such circumstances it was found sufficient to render the solution alkaline before distilling, and thus prevent any of the phenol distilling.

SUBSTANCE	PERCENTAGE ACTUALLY PRESENT	PERCENTAGE FOUND		
1. Iodine, 0.5 per cent . . . . .	7.98	7.95	7.95	7.98
2. Caraway oil, 1 per cent. . . . .	4.57	4.57		
	6.24	6.245		
	8.60	8.56		
3. Iodoform, 1 per cent . . . . .	8.07	8.06	8.07	
4. Amyl nitrate, 4 per cent. . . . .	8.07	8.06	8.06	
5. Chloral hydrate, 6 per cent. . . . .	8.16	8.06	8.00	
Chloral hydrate, 1 per cent. . . . .	8.16	8.08	8.17	
6. Formaldehyde, 1 per cent . . . . .	13.24	13.20	13.15	
7. Acetone, 2 per cent. . . . .	8.07	8.07	8.04	
8. Phosphorus, 0.5 per cent . . . . .	7.79	7.79	7.80	
9. Phenol, 5 per cent. . . . .	8.07	8.07	8.06	
10. Vanillin, 0.2 per cent. . . . .	9.60	9.58	9.61	9.58
11. Arsenic oxide, 0.1 per cent. . . . .	8.07	8.04	8.06	
Arsenic iodide, 0.05 per cent. . . . .	8.07	8.08		
12. Mercuric chloride, 0.1 per cent . . . . .	8.07	8.08	8.03	
Mercuric iodide, 0.05 per cent. . . . .	8.07	8.06	8.07	
13. Pyridine, 1 per cent. . . . .	9.46	9.35	9.41	9.45

<sup>1</sup> *This Journal*, 1922, 5: 538.

*Acetaldehyde*, if present, will vitiate the alcohol results, but it is not removed by any of the methods suggested in this report. Of the methods proposed, oxidation with alkaline silver nitrate promises best results, and studies as to the best conditions for accomplishing this are under way.

So far as can be ascertained no other substances which might interfere are ever present in alcohol mixtures of drugs.

The tabulation of results to substantiate each of the steps mentioned is shown on the preceding page.

All determinations were carried out as directed in the method and in the comments which were given.

#### RECOMMENDATIONS<sup>1</sup>.

The writer wishes to emphasize what has already been stated by Murray about the need for revising the alcohol tables.

The combined method, as now formulated, would read as follows:

#### ALCOHOL IN DRUG PRODUCTS.

Introduce into a suitable flask of about 250 cc. capacity a measured volume of the preparation not exceeding 100 cc. at 20°C. and containing not more than 10 cc. of absolute alcohol. If less than 100 cc. is taken, add sufficient water to make 100 cc. Place the flask in a vertical position and connect by means of a spray trap to an upright condenser, the end of the adapter extending at least one-half inch into the neck of a graduated 50 cc. flask used as a receiver. Pack a little cotton loosely in the mouth of the flask around the stem of the adapter to prevent air currents. Heat the flask carefully so that no charring occurs, and distil at a moderate rate—not less than 25 minutes for the 50 cc. distillate. Discontinue the distillation when the receiver is filled nearly to the mark. Bring to a temperature of 20°C., fill to the mark with water at the same temperature, and mix thoroughly. Allow to stand at the temperature of the balance for at least 1 hour, and weigh. Calculate the specific gravity and determine the index of refraction, ascertaining the percentage of alcohol by volume in the distillate from the reference tables, Nos. 7 and 8<sup>2</sup>. From the percentage of alcohol found in the distillate calculate the percentage in the preparation.

If frothing occurs during distillation, add a few grams of tannin, or hydrolyze with dilute sulfuric acid equivalent to 5 cc. of concentrated acid, heating very gently at first and finally increasing the heat and distilling as usual.

If the preparation contains a large quantity of solids, it may be necessary to dilute further. In that case, distil 100 cc. and use as the material for a redistillation.

If the preparation contains *iodine*, add zinc dust in sufficient quantity, but do not begin distillation until after the reduction is complete.

If the preparation contains *glycerin*, take for distillation such a quantity that the residue in the flask at the end of distillation shall not contain more than 50 per cent of glycerin. If this is not possible, redistil the distillate.

If the preparation contains a *volatile acid*, neutralize with sodium hydroxide or add an excess of magnesium oxide and distil. If it contains *phenol*, add an excess of sodium hydroxide and distil. If it contains a *volatile base*, neutralize with dilute sulfuric or phosphoric acid before distillation.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 76.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 464-505.

If the preparation contains *camphor*, *ether chloroform*, *amyl nitrite*, *benzaldehyde*, or a *volatile oil*, dilute the portion to be distilled, if necessary, so that the alcohol content is not more than 15 per cent by volume; saturate with sodium chloride; and shake with about 20 cc. of petroleum ether in a separatory funnel. Draw off the lower alcoholic salt solution into a second separator and repeat the extraction as before. Draw off the lower alcoholic salt solution into the distilling flask. Wash the two portions of petroleum ether successively with 10 cc. of saturated salt solution, add the aqueous layer to the contents of the distilling flask, and distil. If the presence of extractives renders the shaking out difficult or if for any other reason this is undesirable, the preparation may be distilled as usual, and the distillate may be saturated with salt and shaken out with petroleum ether.

If the preparation contains *iodoform*, add 5 cc. of chloroform and proceed as for the latter.

If the preparation contains *chloral hydrate*, add an excess of strong sodium hydroxide to convert the chloral to chloroform. Stopper the flask, allow to stand 30 minutes, and proceed as for chloroform. If the amount of chloral is high, it may be necessary to shake out more than twice.

If the preparation contains *formaldehyde*, add 50 cc. of U. S. P. hydrogen peroxide solution and an excess of sodium hydroxide, heat on the water bath until formaldehyde is decomposed (effervescence ceases), and distil.

If the preparation contains *acetone*, add an excess of benzaldehyde (5 cc. is usually sufficient) and 10 cc. of 20 per cent sodium hydroxide, and heat on the water bath under a reflux for 30 minutes. Cool, and treat as for benzaldehyde by extracting with petroleum ether. Filter, wash, and distil as usual.

## REPORT ON ARSENICALS—METHOD FOR THE DETERMINATION OF ARSENIC IN SODIUM CACODYLATE.

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

In accordance with last year's recommendation<sup>1</sup> that methods for the determination of arsenic in sodium cacodylate be further studied, the preliminary work was continued. A thorough review of the literature on the subject of arsenicals was made by P. W. Morgan of the Chicago Food and Drug Inspection Station. The method of Norton and Koch<sup>2</sup>, modified by A. J. Ewins<sup>3</sup>, was selected as a result of this study.

This method is an estimation of organically combined arsenic employing a sulfuric acid digestion with the aid of starch to reduce the arsenic. Ewins investigated a series of arsenicals containing both aliphatic and aromatic radicals in the same compound, but sodium cacodylate was not included in his investigations.

For the work this year a product labeled "Sodium Cacodylate U. S. P. IX" was purchased. The sample was examined by U. S. P. assay and was found to be sufficiently pure for the purpose. In addition, a sample of cacodylic acid was prepared from this product by neutralizing the salt and finally crystallizing from chloroform.

<sup>1</sup> *This Journal*, 1925, 8: 510.

<sup>2</sup> *J. Am. Chem. Soc.*, 1905, 27: 1247.

<sup>3</sup> *J. Chem. Soc., Trans.*, 1916, 109: 1355.

The method in detail follows:

REAGENTS.

- (a) *Sulfuric acid, strong.*
- (b) *Starch.*
- (c) *C. P. potassium sulfate.*
- (d) *Sodium hydroxide solution, 1 + 1.*
- (e) *C. P. sodium bicarbonate.*
- (f) *0.1 N iodine solution.*

DETERMINATION.

Transfer 0.2 gram of the sample, accurately weighed, to a Kjeldahl flask. Conduct a blank, using the same quantities of reagents. Add 10 grams of potassium sulfate, 0.3 gram of starch, and 20 cc. of strong sulfuric acid. Digest over a low flame until frothing ceases. Continue the digestion 4 hours, or until colorless. Cool, dilute with water, and transfer to a 500 cc. Erlenmeyer flask. Add sodium hydroxide solution slowly until alkaline to litmus paper and acidify with sulfuric acid. Place the flask in water until thoroughly cooled, add 5 grams of sodium bicarbonate, and titrate with 0.1 N iodine solution.

One cc. of 0.1 N iodine solution is equivalent to 0.00375 gram of arsenic or 0.008 gram of anhydrous sodium cacodylate.

The results of analyses obtained by P. W. Morgan and C. K. Glycart are given in the table.

ANALYST	SAMPLE	METHOD	ARSENIC	ANHYDROUS SODIUM CACODYLATE	U. S. P. SODIUM CACODYLATE CALCULATED ON BASIS OF 72 PER CENT ANHYDROUS SODIUM CACODYLATE
C. K. Glycart	Sodium Cacodylate— U. S. P.	Proposed	<i>per cent</i> 33.69	<i>per cent</i> 71.92	<i>per cent</i> 99.9
			33.77	72.08	100.1
P. W. Morgan	Sodium Cacodylate— U. S. P.	Proposed	33.57	71.65	99.51
			33.66	71.85	99.79
		U. S. P. Assay		71.2 71.2	98.89 98.89
				71.00	98.61
	Cacodylic Acid— Anhydrous	Proposed	53.52	98.55	
		U. S. P. Assay		99.35	

It appears that the proposed method yields highly favorable results. The method is considered of value because the active ingredient is determined.

The associate referee is of the opinion that the method is based on sound principles; the arsenic in organic combination is readily reduced by the sulfuric acid-starch digestion and titrated with standard iodine.

Thanks are due to Morgan for assistance in this work.

#### RECOMMENDATION<sup>1</sup>.

In view of the satisfactory results obtained in this investigation, it is recommended that the method for the determination of arsenic in sodium cacodylate submitted in this report be adopted as a tentative method.

### REPORT ON CAMPHOR AND MONOBROMATED CAMPHOR— THE ESTIMATION OF CAMPHOR IN PILLS AND TABLETS<sup>2</sup>.

By ELGAR O. EATON (U. S. Food and Drug Inspection Station, San Francisco, Calif.), *Associate Referee*.

Since camphor is a physiologically active substance of considerable importance, it would seem to be desirable to have a method suitable for its determination in pills and tablets. The usual dose of camphor is rather large, averaging 0.2 gram by the mouth and 0.1 gram hypodermically. This is fortunate because advantage can be taken of this fact to use an optical method of assay. Many valuable data are available in the literature to show its optical rotation, in varying concentrations, in benzene and in other solvents.

Landolt<sup>3</sup>, Foerster<sup>4</sup>, and Rimbach<sup>5</sup> have done noteworthy work on this subject. Foerster devised a method for determining camphor in celluloid, which gave good results. More recently Edwin Dowzard<sup>6</sup> published a method applicable to pills and tablets based on principles similar to those of Foerster. The late Gail H. Arner<sup>7</sup>, former associate referee, proposed that an attempt be made to adapt the U. S. P. Camphor Liniment assay<sup>8</sup> to this problem. He further proposed to determine camphor by a method suggested by E. K. Nelson<sup>9</sup>.

#### ADVANTAGES OF NEW METHOD.

The observations of the associate referee are that none of these methods offers any advantage over the method proposed in this report, but that they do contain disadvantages. The referee's method is quantitative and

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 76.

<sup>2</sup> Presented by E. K. Nelson.

<sup>3</sup> *Ann.*, 1877, 189: 334; *Ber.*, 1888, 21: 204.

<sup>4</sup> *Ber.*, 1890, 23: 2981.

<sup>5</sup> *Z. physik. chem.*, 1892, 9: 698.

<sup>6</sup> *J. Ind. Eng. Chem.*, 1914, 6: 489.

<sup>7</sup> *This Journal*, 1922, 5: 544.

<sup>8</sup> U. S. Pharmacopeia IX, 233.

<sup>9</sup> Personal communication to Arner and Eaton

requires no complicated apparatus. Small volumes of distillate suffice to remove all the camphor. About 2 hours is required for a determination.

Two samples were submitted to collaborators for work during 1925. Sample 1 was pure refined Japanese camphor in 1 ounce blocks, pulverized by aid of alcohol, air and desiccator dried. Sample 2 was a commercial tablet, coated, and containing, according to the manufacturer's claims, 64.8 milligrams of camphor per tablet.

The method follows:

Take a sufficient quantity of the powdered substance, accurately weighed, to contain approximately 2 grams of camphor. Transfer to a 400 cc. round-bottom Pyrex flask. Add 10 cc. of benzene to the flask. (It is necessary to add the benzene to the distilling flask to insure solution of the camphor and subsequent separation, in a physical state, whereby it is readily carried over by steam.) Add 10 cc. of water and connect the apparatus for steam distillation. Use an 8 or 12 inch bulb condenser, well cooled, and have the outlet reach to the bottom of a 200 cc. flask. Distil with steam, collecting the benzene and about 100 cc. of aqueous distillate. Disconnect and thoroughly wash out the condenser with 5 cc. of alcohol. This can be conveniently accomplished by slowly draining the alcohol into the condenser from a pipet. Follow this washing by an additional washing in the same manner, using 10 cc. of benzene. Add all the washings to the distillate. Saturate the distillate with sodium chloride and add sufficient dilute sulfuric acid to insure acidity. Transfer to a separatory funnel and draw off the aqueous layer into a second separator. Rinse the original receiver with 10 cc. of benzene and add to the aqueous layer in the second separator. Shake, separate, and shake once more with 10 cc. of benzene. Combine benzene and shake with 10 cc. of aqueous salt solution containing sufficient sodium carbonate to insure alkalinity. Separate and shake the washings with 10 cc. of benzene. Transfer the combined benzene solutions of camphor to a 50 cc. volumetric flask and make up to mark. Filter into a 200 mm. polariscopic tube, using a water-jacketed tube, if necessary, in order to maintain a constant temperature. Read at 20°C., using a bichromate filter. Make an average of ten readings. Correct the reading for the zero point.

Calculate as follows<sup>1</sup>:

$$C = 2.4683 \frac{\alpha}{L} - 0.01747 \sqrt{L}$$

Where  $\alpha$  = observed rotation in angular degrees, and

$L$  = length of tube in decimeters.

Divide the value of  $C$  by 2 to find the number of grams of camphor in 100 cc. of benzene. The result equals the number of grams of camphor present in the sample used.

The collaborators reported as follows:

COLLABORATOR	SAMPLE 1	SAMPLE 2
	<i>percentage by weight</i>	<i>milligrams per tablet</i>
D. McIntire . . . . .	98.8	49.6
E. O. Eaton . . . . .	99.0	50.0
E. F. Kinney . . . . .	99.2	48.6 50.4
A. W. Hanson . . . . .	99.0 97.0	51.1 49.2

<sup>1</sup> Landolt. *Optical Rotation of Organic Substances*, 2nd ed., 1902, p. 498.

## CONCLUSIONS.

The collaborative results show that this method is of considerable value and that fair results are obtained when all the conditions prescribed are adhered to.

It is recommended that this method be adopted as tentative without further work<sup>1</sup>.

## REPORT ON CHAULMOOGRA OIL.

By L. E. WARREN (American Medical Association, Chicago, Ill.<sup>2</sup>),  
*Associate Referee.*

In the report on chaulmoogra oil last year<sup>3</sup>, the results of considerable experimental work carried out by the collaborators and the associate referee were recorded. The studies included determinations of the specific gravity, specific rotation, iodine absorption number, saponification number, acid value, index of refraction, and viscosity on several specimens of oil. The constants of mixtures of pure chaulmoogra oil and castor oil were determined, and some tests were carried out on the partial solubility of the pure oil in alcohol. In general, the methods used were those of the Association of Official Agricultural Chemists<sup>4</sup> or those official for fixed oils in United States Pharmacopeia IX<sup>5</sup>. With a few exceptions, the results obtained were reasonably uniform, although the work was not completed. The inequalities reported have been removed for the most part by the work this year.

Two of the collaborators of last year did not report any work this year, and the other collaborators, including the associate referee, did not have much time to devote to the subject. The actual work reported at this time, therefore, is not extensive. However, the fact that sufficient studies were made last year on most of the constants of the oil, and the recognition that U. S. P. X would establish legal standards for the identity and purity of the oil justified the limited efforts.

The names of the collaborators reporting work done this year are the following:

Eli Lilly & Co. (Lilly), Indianapolis, Ind.

Parke, Davis & Co. (P. D. & Co.), Detroit, Mich.

L. R. Wagener (L. R. W.), Ann Arbor, Mich.

H. W. Vahlteich (H. W. V.), New York, N. Y.

L. E. Warren (L. E. W.).

In recording some of the work done this year (especially the corrections), the values found have been placed in the gaps of the tables re-

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 77.

<sup>2</sup> Present address: Bureau of Chemistry, Washington, D. C.

<sup>3</sup> *This Journal*, 1925, 8: 515.

<sup>4</sup> *Ibid.*, 1926, 9: 52

<sup>5</sup> Pages 590, 591, 602.

ported last year, although this arrangement necessitated republishing some of the results. It was thought that this was the least troublesome form in which to make comparisons. The corrected results, together with the additions, are given in Table 1.

TABLE 1.  
*Analyses of chaulmoogra oil by several collaborators.*

COLLABORATOR	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER		
					(a)	(b)	(c)
C. L. C.	0.9523			204.6	31.72		
M. C. K.			104.8 104.9 105.3	200.9 201.1			
McG. & W.	0.9523	43.70	99	198	30.82	30.85	
H. W. V.	0.9561	49.20	101.1	204.70	31.74	31.56	31.38
L. E. W.	0.9520 0.9520	51.41 51.50	102.60 102.55 102.48	203.72 202.52 203.34	30.77	30.45	
C. L. C *	0.9522			199.5	31.82		
M. C. K.			104.7 104.5 104.3	196.2 196.9 196.7			
McG. & W.	0.9520	44.90	99.6	196	29.48	28.8	
H. W. V.	0.9544	50.10	101.7	204.05	30.08	29.82	29.64
L. E. W.	0.9515	50.52	101.16 101.06	198.68	29.09	29.13	
C. L. C.	0.9537			204.9	27.00		
M. C. K.			98.93 98.78 98.78	202.2 202.0 202.6			
H. W. V.	0.9532 0.9530	55.30 55.40	95	207.6 208.3	26.36 26.60	26.88 26.90	25.90 26.00
L. E. W.	0.9538	54.70 56.52	98.60 99.51 96.81	204.75 205.43 203.12	26.18 26.20	25.92 25.63	
L. E. W.	0.9501	51.26	101.79	201.35	16.57	16.84	
L. R. W.	0.9498	50.07	99.83	196.3	13.99		
H. W. V.		50.0		201.0	18.70		

\*Repetition of initials denotes different samples.

Other corrections and additions to the findings for last year are given in Tables 2 and 3.



TABLE 2.

*Analysis of a mixture of chaulmoogra oil and castor oil.*

(Sample D. Chaulmoogra oil 90, castor oil 10.)

COLLABORATOR	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), IN DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER		
					(a)	(b)	(c)
McG. & W.	0.9557	55.3	95.98	186.86	23.3	23.7	
H. W. V.		50.02	94	202	24.34	26.55	
L. E. W.	0.95418	51.50	95.34	201.78	23.92	23.33	
U. S. P. X Standards	0.9500	43-60	98-104	198-213		10-25	

TABLE 3.

*Analysis of a specimen of chaulmoogra oil and castor oil.*

(Sample E. Chaulmoogra oil 80.95, castor oil 19.05.)

COLLABORATOR	SPECIFIC GRAVITY AT 25°/25°C.	POLARIZATION (20°), in DEGREES	IODINE NUMBER	SAPONIFICATION NUMBER	ACID NUMBER (b)
C. L. C.	0.9526			199	20.55
M. C. K.			100.3 100.8 100.6 101.0	195.4 195.2 196.0 195.7	
L. R. W.	0.9469	51.13	101.74	198.1	10.83
L. E. W.	0.9524	47.55	97.49 97.51	197.1 201.3	19.70 19.38
U. S. P. X Standards	0.9500	43-60	98-104	198 213	10-25

In the work completed last year, it was observed that when chaulmoogra oil was mixed with considerable proportions of castor oil the sophistication apparently could not be detected by the methods and standards given in U. S. P. X, but sufficient evidence was not available to establish the observation as being without exceptions. The work this year has been conducted with the view to settling this phase of the problem.

#### ALCOHOL SOLUBILITY.

Since the alcohol solubility was considered to be of considerable importance, the aid of three dealers in chaulmoogra oil was sought. Each of the dealers was requested to subject such commercial samples as were available to the alcohol-solubility test and to report the findings. Two

of the firms submitted their reports, and the third expressed a willingness to collaborate as soon as the opportunity was offered by further importations of the oil. The results reported by the manufacturers are given in Tables 4 and 5.

TABLE 4.  
*Eli Lilly & Co.*

SAMPLE	ALCOHOL SOLUBLE	ACID VALUE
	<i>per cent</i>	<i>per cent</i>
1	17.5	42.4
2	11.8	30.2
3	10.2	33.2
4	8.6	26.9
5	8.3	26.96
6	8.0	26.2
7	None	5.85

TABLE 5.  
*Parke, Davis & Co.*

SAMPLE	SPECIFIC GRAVITY	OPTICAL ROTATION	SOLUBILITY IN ALCOHOL	FREE FATTY ACID <sup>1</sup> CALCULATED AS CHAULMOOGRIC ACID IN ORIGINAL SPECIMEN
		<i>degrees</i>	<i>per cent</i>	<i>per cent</i> <sup>1</sup>
1	0.9540	50.67	6.4	7.68
2	0.9542	52.0	7.6	10.53
3	0.9548	53.67	None	2.54
4	0.9564	52.0	None	2.63

It was pointed out by the manufacturers that the alcohol-soluble matter had a high acidity and that it consisted, for the most part at least, of the free fatty acids of the oil. That is, the free fatty acids of chaulmoogra oil are quite soluble in alcohol, while the combined glyceryl esters are not. This conclusion had been suspected by the associate referee during the work last year, and it had been decided to determine the acid number and the iodine number of the alcohol-soluble portions. These tests were carried out this year by the associate referee on the alcohol-soluble portion of several samples. The findings are recorded in Table 6. The iodine number and the acid number of castor oil and of chaulmoogra acid are included for comparison.

TABLE 6.

*Some constants of the alcohol-soluble portions of the oil compared with the same constants from the original oil.*

SAMPLE	IODINE NUMBER	ACID NUMBER	ACID NUMBER, ORIGINAL OIL	IODINE NUMBER, ORIGINAL OIL
A	98.54	124.0	30.45	102.5
B	99.12	107.07	29.13	101.1
C	94.24	119.68	25.77	98.3
D*	93.71	74.71	23.96 (mixture)	95.3
E†	92.61	49.87	19.54 (mixture)	97.5-101
F	99.65	105.54	16.84	101.8
G‡	99.67	116.18	24.26	103.3
Castor oil			1.57	84.35
Theory for fatty acids from chaulmoogra oil	103.2	215		

\* Chaulmoogra oil 90, castor oil 10.

† Chaulmoogra oil 80/95, castor oil 19.05.

‡ Authentic chaulmoogra oil.

#### DISCUSSION.

It will be noted that the acid number of the original oil is only a small fraction of the acid number of the alcohol-soluble portion of the same oil. This indicates that most of the free fatty acids are dissolved out of the oil by alcohol. The laboratory specimens containing castor oil are indicated by the lowering of the iodine and acid numbers of the alcohol-soluble portions, although the lowering in the iodine values is not marked. Earlier studies had shown that specimens should be suspected which show an abnormally high viscosity and a high solubility in alcohol.

Last year three methods for determining the acid number of the oil were suggested. The one in which carbon tetrachloride was used as a solvent was found to have no advantages over the U. S. P. X method. The official method of the A. O. A. C.<sup>1</sup> for fixed oils was suggested. Since the method calls for large samples of the oil (10 grams as modified), only one collaborator tried it. It appears to possess no advantages over the U. S. P. X method, and as it is extravagant in the quantity of sample required, it was not further considered. The U. S. P. X method is satisfactory.

From the results of the tests, it is believed that the presence of any considerable proportions of castor oil in chaulmoogra oil may be detected

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 293.

by the viscosity of the oil coupled with a determination of the proportions soluble in alcohol. If the acidity of the original oil is high, the fraction soluble in alcohol should be separated, and its acid number and the iodine absorption number of the alcohol-soluble portion should be taken.

#### CONCLUSIONS.

It is concluded from the experimental work carried out in this series of collaborative studies that a specimen of chaulmoogra oil which conforms to the standards required by U. S. P. X is probably genuine and of good quality. However, to be assured of this, additional tests, such as viscosity, loss or gain on heating, and proportion soluble in alcohol are necessary.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the U. S. P. X methods for determining optical activity, iodine absorption number, saponification number, and acid number of fixed oils be made official for chaulmoogra oil.

(2) That the test for solubility in alcohol be adopted as tentative.

(3) That the U. S. P. IX method for viscosity be adopted as tentative.

(4) That the Lifschutz color test for chaulmoogra oil be adopted as tentative.

#### REPORT ON CHLORAMINE PRODUCTS.

By LLEWELYN JONES<sup>2</sup> (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

In accordance with the recommendation that the subject of chloramine products be studied during the coming year, a review of the literature on this subject was made. In 1923 W. H. Heath<sup>3</sup> presented a preliminary report on the methods for the quantitative determination of chloramine-T and dichloramine-T without collaborative work.

For the work this year it was planned to submit to collaborators samples and directions for the quantitative analysis of chloramine-T and dichloramine-T. A sample of chloramine-T obtained from a reputable manufacturer was analyzed by the associate referee, and results of 99.11 and 98.86 per cent were obtained. However, when it was found that U. S. Pharmacopeia X had incorporated essentially the same methods, and that the methods are considered to be satisfactory, further work on the quantitative methods was not deemed advisable.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 77

<sup>2</sup> Presented by F. L. Elliott.

<sup>3</sup> *This Journal*, 1923, 7: 34.

Within recent years, owing to the fact that compounds containing chloramine have been used for disinfecting purposes by the dairymen, its presence in small quantities has occurred in milk. It is suggested that methods for its detection be studied under the subject of Preservatives in Foods.

J. T. Keister<sup>1</sup> has published an article on the detection of chlorine in milk. The method consists of a colorimetric estimation of chlorine and the use of a starch-iodide reagent, as described by Rupp<sup>2</sup>. No active work relating to this method was done this year by the associate referee.

#### RECOMMENDATIONS<sup>3</sup>.

It is recommended—

- (1) That the study of chloramine products as a drug be discontinued.
- (2) That the subject be taken up by the Referee on Preservatives with the suggestion that the Rupp, and any other methods available for the detection of chloramine in small quantities, be given consideration.

#### REPORT ON CHLOROFORM AND CARBON TETRACHLORIDE IN DRUG PRODUCTS.

By H. O. MORAW (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

Last year low collaborative results were obtained on samples of dilute aqueous alcoholic solutions of chloroform prepared and sent out by the associate referee<sup>4</sup>. The first step in studying the method this year, therefore, was directed toward obtaining more complete recovery. This study included (a) refluxing after standing overnight with the reagent, and (b) heating in pressure bottles to complete the reaction. (The use of pressure bottles was suggested by C. K. Glycart, U. S. Food and Drug Laboratory, Chicago, Ill.) The method was tried on carbon tetrachloride, which is becoming widely used as a vermifuge. While refluxing samples which had stood overnight with the reagent, the odor of escaping carbon tetrachloride was detected at the top of the rather long, spiral condenser which seemed to be conclusive evidence that the reaction was incomplete. There would also be a loss if the flask were heated as previously directed, as the cork would pop out.

Table I shows the results of the experimental work carried out on the same samples. The same standard solutions and reagents were used, and the methods of refluxing after standing overnight and heating in pressure bottles were applied.

<sup>1</sup> *Am. J. Pub. Health*, 1925, 15: 781.

<sup>2</sup> U. S. Dept. Agr. Bull. 1114, 1922.

<sup>3</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 77.

<sup>4</sup> *This Journal*, 1925, 8: 528.

TABLE 1.

*Chloroform and carbon tetrachloride by saponification with and without pressure bottles.*

GRAMS PER 100 CC. IN SAMPLE	GRAMS PER 100 CC. FOUND BY REFLUXING					GRAMS PER 100 CC. FOUND BY PRESSURE BOTTLES	
	Chloroform			Carbon Tetrachloride		Chloroform	Carbon Tetrachloride
	40 min.	1.5 hrs.	1 hour	1 hour	3 hours	3 hours	3 hours
Chloroform 10	9.72 9.76	9.72	9.52 9.54			9.92 10.12	
Carbon Tetrachloride 1				0.89	0.84		0.984 0.992

The directions for the determination were changed to include the use of pressure bottles and also to provide for the evaporation of the alcohol from the aliquot so that the nitric acid added would not be consumed in oxidizing the alcohol. The method is repeated this year in order to include these changes, and a complete description of the collaborative samples is given to make the report fully informative.

## PREPARATION OF COLLABORATIVE SAMPLES.

One hundred grams of U. S. P. chloroform, labeled to contain about 1 per cent of alcohol, was quickly weighed in a 100 cc. flask, 5 milligrams in excess being allowed to compensate for unavoidable evaporation. The neck of the flask was quickly washed with a little alcohol, and the contents were rapidly transferred to a liter graduated flask, then made to volume with alcohol and 200 cc. of water. This was designated Sample No. 1. Sample No. 2 was made by pipetting 100 cc. of No. 1 into a flask and making to 1 liter with alcohol and 300 cc. of water. Sample No. 3, carbon tetrachloride, was prepared by weighing rapidly 10 grams of highest purity carbon tetrachloride in a 50 cc. flask, quickly transferring with the aid of alcohol, and making up to 1 liter with alcohol and about 30 cc. of water. These samples were stored in brown bottles stoppered with corks covered with tinfoil. The collaborative samples were sent in brown tincture-mouth bottles also stoppered with corks covered with tinfoil. The tops of the bottles were covered with plaster of Paris tied over tightly with cheese cloth, and, after drying, dipped into warm glue.

## REAGENT AND SUPPLIES.

*Alcoholic potassium hydroxide.*—Dissolve 30 grams of potassium hydroxide in 30 cc. of water. Cool, and add methyl alcohol to make 100 cc. If more than a trace of chloride is present, it should be determined and a correction applied to the chloroform determination.

*Pressure bottles.*—Citrate of magnesia bottles, with the clamp stoppers, have been found satisfactory, but they should be tested for leakage under pressure. The regular pressure bottle of 60–100 cc. capacity is to be preferred. The rubber washers on the magnesia bottles do not affect the results.

## DETERMINATION.

Accurately measure 5–10 cc. of the sample, corresponding to 0.005–1 gram of chloroform, into a pressure bottle containing 30 cc. of the alcoholic potassium hydroxide.

Stopper the bottle, mix thoroughly, and set into a vessel containing warm water and bring to the boiling point, maintaining this temperature for 3 hours. Cool, and transfer with water to a 200 cc. volumetric flask. Evaporate an aliquot on the steam bath before a fan to remove the alcohol. Determine the chlorine content of the aliquot by the Volhard method. One cc. of 0.1 *N* silver nitrate = 3.98 mg. of chloroform or 3.846 mg. of carbon tetrachloride. Report results in grams per 100 cc.

TABLE 2.

*Collaborative results on chloroform and carbon tetrachloride.*

COLLABORATOR	SAMPLE NO. 1 10 GRAMS CHCl <sub>3</sub> PER 100 cc.	SAMPLE NO. 2 1 GRAM CHCl <sub>3</sub> PER 100 cc.	SAMPLE NO. 3 1 GRAM CCl <sub>4</sub> PER 100 cc.	PRESSURE BOTTLE
	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	<i>grams per 100 cc.</i>	
Percy Tarver Health Dept. Cleveland, O.	9.756 9.698	0.935 0.911	0.966 0.942	Citrate of magnesia 12 fl. oz.
L. S. Crosby United Drug Co. Boston, Mass.	9.461	0.957	0.917	Citrate of magnesia 12 fl. oz.
Loren Burritt Bureau of Internal Revenue, Washington, D. C.	9.61 9.46	1.098 1.050	1.130 1.014	Citrate of magnesia 12 fl. oz.
Peter Valaer, Jr. Bureau of Internal Revenue, Washington, D. C.	9.38 9.55	1.082 1.050	1.146 1.146	Citrate of magnesia 12 fl. oz.
Robert V. Pegau Bureau of Chemistry New York	9.708	0.962	0.981	Regular pressure 130 cc.
G. E. Mallory Bureau of Internal Revenue, Washington, D. C.	9.55 9.46	1.018 0.971	1.000 1.000	Citrate of magnesia 12 fl. oz.
H. Wales Bureau of Chemistry Washington, D. C.	9.437 9.389 9.487*	0.9297 0.9263 0.9253	0.8861 0.9662 0.8861 0.7250*	Special bottles rubber stopper 100 cc.
H. O. Moraw	10.12 9.92	0.992 0.992	0.984 0.992	12 fl. oz. magnesia bottles for CHCl <sub>3</sub> ; 60 cc. clamp crown cork style for CCl <sub>4</sub> .
E. L. Henderson Univ. of Tenn., College of Medicine, Memphis, Tenn.	9.49 9.37	0.935	1.21 1.15	Ground glass stoppered bottle, 325 cc.
S. Webster Dodge, Lehn & Fink Bloomfield, N. J.	9.22 9.23	0.771 0.764	0.822 0.822	Special bottle, 120 cc. Rubber rings.

\*Samples stood overnight without heating.

## COMMENTS BY COLLABORATORS.

*Percy Tarner:* An inverted wire basket with cloth tied around it was put over the pressure bottles after one of them had burst. By reporting grams per 100 cc. any error is magnified 40 times. After the addition of the excess of silver nitrate, the volume was made to 200 cc., and an aliquot of the filtrate was used to titrate.

*E. L. Henderson:* The pressure bottle with sample and reagent was placed in a constant level bath in cold water. Heat was applied, and the time was noted when the water boiled. The bottles were about two-thirds immersed. In the titration the red color which persisted one minute was taken as the end point.

*H. Wales:* Regular pressure bottles, which we first tried, leaked around the rubber gasket. When fitted with rubber stoppers wired in place, they exploded. Some were then made from combustion tubing, closed with rubber stoppers wired in place. These were satisfactory. Heating was accomplished by placing the bottles in the steam bath.

## DISCUSSION OF RESULTS AND COLLABORATORS' COMMENTS.

The method was intended to apply to the estimation of small quantities of chloroform such as would be obtained in the distillates from liniments and cough medicines, etc., and the collaborative samples were assumed to be comparable to these distillates freed from interfering substances.

The majority of the collaborative results in Table 2 are in agreement, if extremes are eliminated. From a study of the results and comments by collaborators and others, including A. E. Paul and C. K. Glycart of the U. S. Food and Drug Laboratory, Chicago, Ill., it is concluded that the method in its essentials is satisfactory. It has been revised, however, to include the points suggested by this study in the following details:

- (a) Size of pressure bottle.
- (b) Standing in pressure bottle before heating.
- (c) Starting the heating in water at room temperature.
- (d) Protection against bursting of pressure bottles.
- (e) Requiring a blank on the reagents.
- (f) Gravimetric estimation optional.
- (g) End point in Volhard's method according to U. S. P. IX, p. 570.

It was noticed that small variations in the end-point readings cause considerable variation in the results. Thus 0.2 cc. variation on the 10 gram per 100 cc. sample is multiplied by 125 to obtain grams per 100 cc. The chloroform equivalent is 0.1 gram, or 1 per cent on 10 grams. The same variation on the smaller sample is equivalent to about 1.6 per cent. This multiplication of error may be kept to the minimum by using larger aliquots for the precipitation, or a larger portion of the saponified sample may be used for the gravimetric estimation, thus reducing the error.

## LIMITS OF ACCURACY.

The limits of accuracy of the method depend upon the care of the worker in conducting the saponification and the final estimation. With



good pressure bottles and accurately standardized solutions, an experienced analyst will get within 0.3–0.5 per cent of the quantity present.

### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the following method for the determination of chloroform and carbon tetrachloride be adopted as tentative:

#### ESTIMATION OF CHLOROFORM AND CARBON TETRACHLORIDE.

##### REAGENTS.

(a) *Alcoholic potassium hydroxide*.—Dissolve 30 grams of potassium hydroxide in 30 cc. of water. Cool, and dilute to 100 cc. with methyl alcohol.

(b) *Silver nitrate*.—0.1 N solution.

(c) *Ammonium or potassium thiocyanate*.—0.1 N solution.

(d) *Nitric acid*.—Free from the lower oxides by diluting concentrated nitric acid with water (4 + 1) and boiling until colorless.

(e) *Ferric ammonium sulfate indicator*.—Saturated solution of ferric ammonium sulfate  $(\text{NH}_4)_2\text{SO}_4 \cdot (\text{Fe}_2(\text{SO}_4)_3 \cdot 24\text{H}_2\text{O})$ .

##### PREPARATION OF SAMPLE.

*Chloroform in medicinal preparations*: Separate by distillation on the steam bath into alcohol in a volumetric flask in cold water. Make up to volume at 20° with alcohol, and stopper.

*Carbon tetrachloride in capsules*: Ascertain the gross weight of a representative number of capsules. Open the capsules with a pointed knife and tweezers under a small quantity of alcohol. Transfer the alcoholic solution to a volumetric flask, washing the capsules free from carbon tetrachloride. Weigh the dried, empty capsules and calculate the average net contents. Fill the flask to the mark with alcohol at 20°C., and stopper.

*Chloroform U. S. P. and carbon tetrachloride*: Weigh a suitable quantity into a volumetric flask and make up to volume at 20°C. with alcohol, or weigh accurately about 1 gram directly into a 1 or 2 cc. ground-glass, stoppered weighing bottle. (Such as used for Victor Meyer molecular weight determination.)

##### DETERMINATION.

Transfer 5–10 cc. of the alcoholic solution of the sample accurately measured at 20°C., corresponding to 0.005–1 gram, or weigh accurately 0.1–1 gram of chloroform or carbon tetrachloride into a 1 cc. ground-glass, stoppered weighing bottle (the Victor Meyer molecular weight weighing bottle is satisfactory), and transfer to a 60–75 cc. pressure bottle, containing 30 cc. of the alcoholic potassium hydroxide. Remove the stopper from the weighing bottle by working it out with a glass rod while submerged in the reagent. Wash off the rod with a little alcohol. Conduct a blank in another pressure bottle with the same amount of reagent. Stopper the bottle and mix thoroughly. Let stand 1 hour with occasional shaking. Set into a bath of water at room temperature. To protect from bursting, invert a wire basket over the bottle and cover with a towel. Gradually heat the water. Maintain at boiling temperature for 3 hours. Cool, transfer with water to a 200 cc. volumetric flask. Wash out the weighing bottle with a thin

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 77.

specially made spout attached to the wash bottle. Complete the volume at 20°C. and evaporate the alcohol in a 400 cc. beaker on the steam bath from an aliquot taken at 20°C. Determine the chloride by the Volhard method or gravimetrically. Determine the end point in the titration as for standardizing the sulfocyanate solution<sup>1</sup>. Deduct the chloride found in the blank. One cc. of 0.1 *N* silver nitrate = 3.98 mg. of chloroform or 3.846 mg. of carbon tetrachloride.

(2) That the method be submitted next year with samples of pure chloroform for collaborative study with a view to making it official.

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#### REPORT ON IPECAC ALKALOIDS<sup>2</sup>.

By A. R. BLISS, JR. (College of Medicine, University of Tennessee, Memphis, Tenn.), *Associate Referee*.

In accordance with the recommendations adopted at the 1924 meeting relating to methods for determining the alkaloidal content of ipecac and its preparations<sup>3</sup>, the five methods given in this report were submitted to collaborative investigation, fluidextract of ipecac being selected for the study. Carefully prepared samples were sent to five collaborators, who were instructed to carry out the following methods:

##### I.—U. S. P. X VOLUMETRIC METHOD<sup>4</sup>.

Follow the U. S. P. X Type Process D<sup>5</sup> by: (1) pipetting 5 cc. of the fluidextract directly into a separator containing some ether; (2) adding about 5 cc. of distilled water; (3) rendering the mixture alkaline by the addition of ammonia T S.; (4) completely extracting the alkaloids by shaking out with successive portions of ether; (5) completing the assay as directed under Type Process A beginning with the words "Shaking out with Acid"<sup>6</sup>; and (6) determining the alkaloids *volumetrically*, using methyl red as the indicator.

##### II.—U. S. P. X GRAVIMETRIC METHOD.

Repeat the U. S. P. X method outlined under Method I, except the final determination of alkaloids, which, in this case, should be determined gravimetrically as described on page 453, U. S. P. X, but allow the solvent to evaporate spontaneously or in a current of air as suggested in Method IV.

##### III.—THE PALKIN-WATKINS METHOD.

Apply to the fluidextract of ipecac the method described by these investigators for fluidextract of nux vomica<sup>7</sup>, substituting ether as the solvent and changing the titration factor to 0.024.

<sup>1</sup> U. S. Pharmacopeia IX, p. 570.

<sup>2</sup> Presented by A. G. Murray.

<sup>3</sup> *This Journal*, 1925, 8: 529.

<sup>4</sup> U. S. Pharmacopeia X, p. 170.

<sup>5</sup> *Ibid.*, 455.

<sup>6</sup> *Ibid.*, 453.

<sup>7</sup> *J. Am. Pharm. Assoc.*, 1924, 13: 694.

IV.—MODIFIED U. S. P. IX METHOD<sup>1</sup>.

Drop 10 cc. of fluidextract of ipecac evenly over the surface of 10 grams of sawdust in a casserole, and *evaporate to dryness in a vacuum desiccator under vacuum*. Transfer the impregnated sawdust to a 250 cc. Erlenmeyer flask and add 100 cc. of ether. Rinse the casserole in which the mixture was evaporated with 6 cc. of ammonia water previously diluted with an equal volume of distilled water, using several portions and adding the washings to the flask. Stopper the flask tightly and shake vigorously every few minutes for 2 hours.

Add 15 cc. of distilled water and shake the flask well; after the sawdust has subsided, filter 50 cc., representing 5 cc. of sample.

Transfer this 50 cc. portion to a 500 cc. separatory funnel and extract the alkaloids completely by shaking out with weak sulfuric acid (10 cc. of 0.5 *N* sulfuric acid for the first shaking, and a mixture of 5 cc. of 0.5 *N* sulfuric acid and 5 cc. of distilled water for the remaining shakings), four or five shakings usually being necessary. Test  $\frac{1}{4}$  cc. of this sulfuric acid washing with potassium mercuric iodide T. S. to insure complete extraction of the alkaloids. Continue the shakings until all the alkaloids have been extracted.

Collect the sulfuric acid washings in a second 500 cc. separatory funnel. Make the acid solution in this second funnel alkaline with ammonia water to liberate the alkaloids. Extract the free alkaloids with ether, about four washings being necessary—50 cc. for the first and 25 cc. for the remaining washings. Filter the ether washings through cotton into a 250 cc. Erlenmeyer flask.

Continue the extractions until the following test is negative: Evaporate 1 cc. of the ether washing to dryness and dissolve in a few drops of dilute hydrochloric acid, testing with potassium mercuric iodide T. S.

When drawing off from one separatory funnel to another, as in making separations, wash the funnel with the fresh solvent.

Evaporate the combined washings, at room temperature, to dryness. (A little air blown into the Erlenmeyer flask will hasten the process.) When the solvent is completely evaporated, add 1 cc. of neutral alcohol to soften the residue. Dissolve the residue in 10 cc. of 0.1 *N* sulfuric acid, applying gentle heat to aid in solution. After the residue has dissolved, titrate the excess acid with 0.02 *N* potassium hydroxide, using methyl red as indicator.

## V.—MECHANICAL EXTRACTOR METHOD.

Apply to the fluidextract the method described by Palkin, Murray, and Watkins<sup>2</sup>. If the alkaloid precipitated upon the addition of ammonia is permitted to agglomerate at the bottom of the extractor jacket, it is difficult to extract. To obviate this difficulty, use the following procedure: Place the aliquot (20 cc.) of the purified ipecac preparation in a separator, add ammonia water in sufficient excess, then 30 cc. of ether. Shake thoroughly and draw the entire contents of the separator into the jacket of the extractor, washing the separator, first with a little water, and then with ether.

NOTE: The apparatus specified in the reference given previously as "Apparatus B, Figure 1" should be used in carrying out this method. If this type of mechanical extractor is not available to collaborators, the Bureau of Chemistry will lend one for a sufficient period to do this work. Collaborators desiring to borrow this apparatus should communicate with the Bureau of Chemistry, Department of Agriculture, attention of Drug Control, Washington, D. C.

<sup>1</sup> Method IV was suggested by M. A. Dittmar of the Research Laboratory of Lehn & Fink. V. O. Mattson reported the method in *The Druggists Circular*, June, 1925. The method is given as described by Dittmar.

<sup>2</sup> *Ind. Eng. Chem.*, 1925, 17: 612.

The results of the analyses of the submitted sample of fluidextract of ipecac obtained by the five analysts are given in Table 1.

TABLE 1.  
*Results of analysis of fluidextract of ipecac.*  
(Grams per 100 cc.)

ANALYST	WATKINS	MORAW	GLYCART	MATTSON	BLISS
I.—U. S. P. X Volumetric Method	2.304 2.314 — Av. 2.309	See "Com- ments" by analysts	1.52	See "Com- ments" by analysts	1.98 1.83 — Av. 1.90
II.—U. S. P. X Gravimetric Method	2.9060* 3.0840* — Av. 2.9950  2.6400† 2.6520† — Av. 2.6160	See "Com- ments"	See "Com- ments"	2.56 2.71 — Av. 2.635	1.29 2.15 — Av. 2.22
III.—Palkin- Watkins Method	2.436 2.424 — Av. 2.43	2.27 2.42 — Av. 2.345	2.14	2.09 2.02 — Av. 2.055	2.41 2.38 — Av. 2.395
IV.—Modified U. S. P. IX Method	1.670 1.680 — Av. 1.675  1.790† 1.800† — Av. 1.795	1.54 0.65 See "Com- ments"	0.36 0.67 See "Com- ments"	1.74	1.71 1.57 — Av. 1.64
V.—Mechanical Extractor Method	2.460 2.484 — Av. 2.472	2.37 2.45 2.30 — Av. 2.375	2.06 (1.92) — Av. 1.99	See "Com- ments"	See "Com- ments"

\* Dried at room temperature.

† Dried at 100°C.

‡ Under this method an aliquot of 25 cc. of the solvent ether was evaporated directly on the steam bath instead of being shaken out with acid and re-extracted with ether.

#### COMMENTS BY ANALYSTS.

*Howard R. Watkins, Drug Control Laboratory, Bureau of Chemistry, Washington, D. C.:*

(1) In our opinion the gravimetric methods are practically worthless.

(2) The U. S. P. IX method evidently fails to extract all the alkaloid. The modified form does not appear to yield any more alkaloid and is open to the objection of the slow process of drying in a vacuum. The ether extract is a beautifully clear solution, so clear in fact that there does not seem to be any need of subsequent shaking with acid and re-extraction with ether.

(3) The U. S. P. X method yields higher results, but the emulsion is particularly difficult to break up, requiring the use of a centrifuge.

(4) The Palkin-Watkins method yields still higher results and the emulsion, while bad, could be broken up without the use of the centrifuge.

(5) The automatic extractor gave the least trouble with emulsions and the highest yield of alkaloids.

(6) The end point of the titration was best in Method IV.

(7) The sample yields emulsions with the immiscible solvents more easily than any other ipecac preparation we have examined. This made the work long and tedious and particularly difficult in the separatory funnel processes.

*Harry O. Moraw, U. S. Food and Drug Inspection Station, Chicago, Ill.:*

1.—*U. S. P. X Volumetric Method:* Could not complete the extraction on any one of three determinations tried because of bad emulsions which would not break. Tried using 1 cc. of 10 per cent ammonia on two of them and 0.3 cc. of 10 per cent ammonia on the other. This variation in the ammonia did not seem to reduce the tendency toward emulsions. The emulsion seemed to be due largely to the addition of the 5 cc. of water, but the sample itself also seemed to be unusual with respect to the amount of solids and the emulsifying tendency.

2.—*U. S. P. X Gravimetric Method:* Did not try for the reasons given under Method 1.

3.—*Palkin-Watkins Method:* The directions for this method are not clear with respect to the amount and concentration of ammonia. The authors direct 1 cc. of ammonium hydroxide, which is generally assumed to be the concentrated (28 per cent ammonia). In their experiments they tried varying amounts of normal, 0.5 N, 8.5 per cent, and 10 per cent, sometimes referring to it as ammonia and sometimes as ammonium hydroxide, which would give different amounts of ammonia. The writer found that 1 cc. of 28 per cent ammonia caused worse emulsions than 2 cc. of 8 per cent ammonia on this sample of ipecac. On samples of belladonna and nux vomica previously analyzed by this method, when using 1 cc. of 28 per cent ammonium the method worked without causing emulsions. The reported results were obtained on the sample with this method when 2 cc. of 8 per cent ammonia was used.

4.—*Modified U. S. P. IX Method:* It is not known why the reported results are so much lower and failed to check. No emulsions—clear end point.

5.—*Mechanical Extractor Method:* The time of extraction was about 2.5 hours. The end point in the titration was obscured by the presence of resins. Some water was carried over by the ether, in each case necessitating re-extraction.

*C. K. Glycart, Food and Drug Inspection Station, Chicago, Ill.:*

(1) None of the results reported is considered satisfactory. Owing to troublesome emulsions, complete extraction was not obtained after 12 extractions in Methods 1 and 3. Even gentle shaking, as in ordinary extraction operation, formed emulsions. For this reason further work was abandoned. Two days was required for the extractions.

(2) In Methods 4 and 5 no appreciable amount of plant material was removed by the dealcoholizing operation. A positive test for glycerin was obtained on the residue.

(3) With regard to Method 4, vacuum desiccation was applied for two days. No emulsions were formed, however, and the low results are not understood.

*V. O. Mattson, Research Laboratories, Lehn & Fink, Bloomfield, N. J.:*

(1) Circumstances prevented us from starting work on the ipecac assays until rather late, and then after having barely started, the bottle containing the material was broken and the sample lost.

(2) Method II produced results of 2.56 and 2.71 per cent. The latter figures are very likely high owing to the fact that extraneous material had been weighed in with the alkaloids.

## DISCUSSION.

*I.—U. S. P. X Volumetric Method:* It is the opinion of the associate referee that this method is decidedly unsatisfactory because of the ease with which very troublesome emulsions form. The average results obtained by three of the five analysts are: (1) 2.309; (2) 1.52; (3) 1.90.

*II.—U. S. P. X Gravimetric Method:* This method, of course, has the same disadvantages and objections as in the case of Method I. However, the average results of the three analysts who reported are interesting, viz., (1) 2.995 (room temperature), 2.696 (100°C.); (2) 2.635; (3) 2.22. Attention is called to the possible effect of heat on the alkaloids as shown by Watkins' average of 2.995 when dried at room temperature and 2.696 when dried at 100°C.

*III.—Palkin-Watkins:* The results obtained by five analysts, viz., (1) 2.43; (2) 2.345; (3) 2.14; (4) 2.055; (5) 2.395, indicate that the method is rather reliable. It yields higher results than Methods I and IV. Although emulsification takes place readily, the emulsion can be more easily broken up than in the case of those formed with Methods I and II.

*IV.—U. S. P. IX Modified:* The results indicate that this method fails to extract the alkaloids completely. The yields were uniformly less than those obtained with the first three methods. The unusually low results obtained by two of the collaborators are not understood. Although no emulsions form, and the end point is best with this method, nevertheless it is decidedly unsatisfactory.

*V.—Mechanical Extractor Method:* At the suggestion of Arthur E. Paul, Referee on Drugs, the mechanical extractor method was carried out only by those analysts in the Food and Drug Inspection Station, Chicago, Ill., and the Bureau of Chemistry, Washington, D. C. Accordingly, but three of the five analysts reported results for this method, viz., (1) 2.472; (2) 2.375; (3) 1.99. (Apparently some slight error is intimated in Glycart's second estimation, which he reported in parentheses.) Although this method gave the highest yield in each analyst's series with Methods I, III, IV, and V, (excepting Method III by Glycart), the average results obtained with Methods II, III, and V, given in Table 2, are interesting.

TABLE 2.  
Average results by Methods II, III, and V.  
(Grams per 100 cc.)

METHOD	AVERAGE YIELD	NO. OF ANALYSTS REPORTING
II.—U. S. P. X Gravimetric. . . . .	2.3731	3
III.—Palkin-Watkins. . . . .	2.2878	5
V.—Mechanical Extractor. . . . .	2.2920	3

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That the collaborative study of ipecac alkaloids be continued.
- (2) That future collaborative study include the Mechanical Extractor Method (Method V), the Palkin-Watkins Method (Method III), and the gravimetric modification of the U. S. P. X assay of ipecac (Method II).

No report on radio activity in drugs and water was given by the associate referee.

## REPORT ON LAXATIVES AND BITTER TONICS.

By H. C. FULLER (Washington, D. C.), *Associate Referee*.

No work has been conducted on the gravimetric method, which was the subject of the report at the meeting in 1924<sup>2</sup>.

Investigations were conducted on a colorimetric method, with the object of developing a procedure that would check with the gravimetric assay. The colorimetric test previously reported was cumbersome, and unless the technique was well understood the results obtained were erratic.

During the work this year it developed that a fairly rapid procedure could be adopted, and that by proceeding along the lines that are followed in making the gravimetric assay, and at the proper time applying the colorimetric reagents, it was possible to obtain results that were consistent with the data obtained with the same specimens assayed gravimetrically.

In the experiments samples of 1 gram each of the powdered drugs were employed, and the readings were checked against those obtained with a product of known gravimetric assay. When running fluidextracts, samples of 5 cc. were taken, and the readings were compared in the same manner.

At present the method of procedure that gives indication of development into a satisfactory test is as follows:

Treat the specimens according to the method previously described<sup>3</sup> to the point where the anthraquinones have been removed from the chloroform by means of 10 per cent sodium hydroxide.

Run the alkaline liquid into a volumetric flask, 100 cc. capacity, and make up to volume with water. By means of a graduated pipet, transfer the solution in portions of 1, 2, 3, 4, and 5 cc. each to separate 50 cc. Nessler tubes and make up to volume

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 77.

<sup>2</sup> *This Journal*, 1925, 8: 536.

<sup>3</sup> *Ibid.*, 537.

with distilled water. Compare the depth of color of the liquid in the respective flasks with that obtained with the control run in the same way, and from the comparisons calculate the percentage of anthraquinones.

The results thus far are too meager to permit of a final approval of the method for submission to collaborators, but it is hoped that by further study the uncertainties in the procedure may be rectified and a set of directions prepared to be submitted for study during the coming season.

It is of interest to note that in pursuing the physiological researches in conjunction with the chemical investigation, it is still apparent that the action of cascara and its extract is in the long run measured by the anthraquinone content. Furthermore, the character of the color is assuming a definite significance and it may eventually enable the chemist to distinguish an extract of pure cascara from one made with the addition or substitution of other anthraquinone-bearing drugs, notably the use of senna siftings instead of cascara bark in the fluidextract.

It is recommended<sup>1</sup> that the committee approve a continuance of the study of this subject.

## REPORT ON MERCURIALS.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

Early in 1925 the attention of the associate referee was drawn to the determination of mercuric chloride in corrosive sublimate tablet triturates. It was found that the methods of Jamieson and Rupp<sup>2</sup> were entirely useless on account of the lactose used in the preparation of the tablets. Even the official method<sup>3</sup> of precipitation by hydrogen sulfide was not so easily carried out as in other mercury preparations. The problem was finally solved by utilizing the lactose as a reducing agent in the same way as formaldehyde is used in the Rupp method.

### METHOD.

#### REAGENTS.

(a) *Potassium iodide solution*.—Dissolve 25 grams of potassium iodide in 50 cc. of water.

(b) *Sodium hydroxide*.—4 per cent solution.

(c) *Acetic acid*.—18 per cent solution.

(d) *Iodine solution*.—0.1 N.

(e) *Sodium thiosulfate solution*.—0.1 N.

(f) *Starch solution*.—1 gram in 200 cc. of water.

(g) *Gum arabic (acacia)*.—5 per cent solution in water.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 77.

<sup>2</sup> *This Journal*, 1925, 8: 538.

<sup>3</sup> U. S. Pharmacopeia X, p. 187.



## PROCEDURE.

Weigh 2 grams of the powdered, well-mixed sample. Dissolve in water and dilute to 100 cc. Divide the solution into two 50 cc. portions and add to each 5 cc. of gum arabic and 25 cc. of the sodium hydroxide solution. Allow to stand in a dark place for one hour. Acidify with 10 cc. of the acetic acid and add 25 cc. of iodine solution. As soon as the mercury is completely dissolved titrate back with sodium thiosulfate solution, using the starch indicator.

One cc. of 0.1 *N* iodine solution is equivalent to 0.01358 gram of mercuric chloride.

## RESULTS OBTAINED BY THE ASSOCIATE REFEREE.

A 2 per cent solution of mercuric chloride containing 2 grams of lactose:

SOLUTION TAKEN cc.	HgCl <sub>2</sub> gram	RECOVERED gram
40	0.08	0.08419
20	0.04	0.0411

A solution of mercuric chloride and sodium chloride containing 0.05 gram of HgCl<sub>2</sub> in each 10 cc.:

10 cc. taken in each case with 2 grams of lactose.

RECOVERED gram
0.0520
0.0543
0.0543

Tablet triturates containing 1-30 grains (2.16 mg.) of mercuric chloride:

Weighed 2 grams of powdered sample.

SAMPLE	RECOVERED per cent	ANALYST
1a	2.82	G. C. S.
2a	2.13	"
2b	2.20	"
3a	2.15	"
3b	2.19	"
4a	2.20	H. D. W.
4b	2.20	"
5a	2.28	"
5b	2.18	"

Preliminary investigations have been made on methods for the estimation of mercury in corrosive sublimate gauze, in mercurous iodide tablets, and in mercurous chloride preparations.

Estimations by the two official methods, electrolysis and precipitation with hydrogen sulfide, have been carefully reviewed and used when possible as standards for the newer and more unfamiliar procedures.

No collaborative work other than indicated has been attempted this year.

The assistance of H. D. Weihe in this work is hereby duly acknowledged with appreciation.

RECOMMENDATION<sup>1</sup>.

It is recommended that the study of analytical methods for mercurials be continued.

## REPORT ON PYRAMIDON.

By WILLIAM RABAK<sup>2</sup> (U. S. Food and Drug Inspection Station, Minneapolis, Minn.), *Associate Referee*.

At the request of the Referee on Drugs, an attempt was made to develop an extraction method for the assay of pyramidon.

For the purposes of this investigation a commercial specimen of pyramidon was obtained and identified by means of its melting point and qualitative tests<sup>3</sup>. The melting-point determination was conducted according to the U. S. P. method<sup>4</sup>. The material was prepared by powdering and drying in vacuo over sulfuric acid for three days. The melting point reported is that actually observed and is uncorrected for emergent stem. The melting was found to begin at 106.5°C., and the product was completely liquefied at 107.5°C., with no apparent decomposition. This result, together with the positive reactions obtained qualitatively, was thought to be sufficient evidence to identify the product positively as pyramidon.

The method proposed is based on the relative solubility of pyramidon in chloroform in ammoniacal solution. Advantage is taken of the fact that pyramidon is easily soluble in dilute hydrochloric acid. Upon the addition of ammonia to the hydrochloric acid solution, the hydrochloride is decomposed, and pyramidon, liberated, is then shaken out with chloroform.

The assay has not been designed as a means of separating pyramidon from certain other chloroform-soluble substances such as alkaloids, antipyrine, etc., but it is offered as a method for the assay of pyramidon powder or tablets of pyramidon.

## METHOD.

Transfer 1 gram of the material to a 100 cc. volumetric flask. Add 60 cc. of normal hydrochloric acid and shake for several minutes to insure complete solution of the pyramidon. Fill to mark with normal hydrochloric acid and filter if not clear. Pipet a 20 cc. aliquot to a separatory funnel. Make distinctly alkaline with ammonia and shake out with 20, 15, 10, 10, and 5 cc. portions of chloroform. Combine the chloroform extractions in another separatory funnel and wash with 2 cc. of water. Filter into a tared beaker through a pledget of cotton saturated with chloroform. Extract the wash water with 5 cc. of chloroform and add this to the combined chloroform extractions.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9, 78

<sup>2</sup> Presented by H. Runkel.

<sup>3</sup> *This Journal*, 1926, 9: 544.

<sup>4</sup> U. S. Pharmacopeia IX, p. 596

Evaporate just to dryness on the water bath with the aid of an electric fan, place in a water oven at 100°C. for 10 minutes, and weigh as pyramidon. Identify the pyramidon by means of its melting point or by qualitative tests, or both.

Five determinations conducted by the associate referee according to the method given gave the following results:

ARRAY NO.	WEIGHT OF PYRAMIDON IN ALIQUOT	WEIGHT RECOVERED	
	gram	gram	per cent
1	0.1000	0.1000	100.00
2	0.1000	0.0993	99.30
3	0.1000	0.1000	100.00
4	0.2000	0.1990	99.50
5	0.2000	0.2000	100.00

#### RECOMMENDATION<sup>1</sup>.

It is recommended that the method given in this report be studied further by collaborators during the coming year.

### REPORT ON THE SEPARATION OF QUININE AND STRYCHNINE.

By F. L. ELLIOTT (U. S. Food and Drug Inspection Station, Boston, Mass.). *Associate Referee.*

In accordance with the recommendation adopted at the 1924 meeting, that the Simmonds method be further studied, a sample of quinine and strychnine was sent out to various collaborators for examination by this method. Some minor additional changes in the method as submitted last year include the removal of excessive alcohol before extraction of total alkaloids, the drying of quinine residue to the anhydrous form, and more specific instructions as to the number of extractions to be made. A method for the titration of the separated alkaloids was also included.

No difficulties were reported by the collaborators except those relating to the separation of solids on evaporation to remove alcohol from the original sample. This difficulty was overcome by diluting the original sample.

The method submitted has been published<sup>2</sup>.

#### COMMENTS OF COLLABORATORS.

*A. Stikarofsky:* The method works well. The alkaloids are quantitatively separated. Absence of glycerin in collaborative sample might affect results. (NOTE: Glycerin in 1924 collaborative sample and results found satisfactory.)

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 78.

<sup>2</sup> *This Journal*, 1926, 9: 53.

*Collaborative results.*

(50 cc. of sample.)

COLLABORATOR	GRAVIMETRIC		VOLUMETRIC	
	Quinine	Strychnine	Quinine	Strychnine
	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>
A. Stikarofsky United Drug Co. Boston, Mass.	0.4122 0.4113	0.0198 0.0195	0.4150 0.4100	0.0195 0.0199
C. A. Herrman Food and Drug Inspection Station New York, N. Y.	0.3892 0.3646	0.0234 0.0249	0.3539 0.3394	0.0208 0.0221
L. A. Salinger Food and Drug Inspection Station Savannah, Ga.	0.3826 0.3985 0.3852	0.0195 0.0208 0.0215	0.3700 0.3819 0.3764	0.0214 0.0214 0.0231
C. K. Glycart Food and Drug Inspection Station Chicago, Ill.	0.4025 0.4034	0.0216 0.0156		
P. W. Morgan Food and Drug Inspection Station Chicago, Ill.	0.4100 0.4150	0.0165 0.0205		
F. L. Elliott	0.4070 0.4204 0.4093	0.0205 0.0197 0.0185	0.3965 0.4023 0.3950	0.0194 0.0207 0.0190
Theoretical	0.4385	0.0200		

*C. A. Herrman:* Strychnine solution after titration gave positive test for quinine, but quinine solution gave no test for strychnine.

*C. K. Glycart:* The chloroform-ether ratio should be included in directions for extraction of mixed alkaloids. Method requires about 4 days. Strychnine results not entirely satisfactory.

*P. W. Morgan:* Amount of citric acid to be added should be specified.

*L. A. Salinger:* Qualitative tests for quinine in strychnine—fluorescence and erythroquin (modified thalleioquin) negative. Strychnine in quinine by bichromate and sulfuric acid and ferricyanide tests negative.

## SUPPLEMENTARY NOTE.

In addition to the collaborative work reported, the associate referee determined the strychnine in the collaborative sample by a method used in the Bureau of Chemistry. This method specifies the separation of the bulk of quinine in the mixed alkaloids by one precipitation with 3-4 cc. of 10 per cent potassium ferrocyanide in 50 cc. of a 5-7 per cent sulfuric acid solution. The precipitate is made alkaline with ammonia, and the strychnine, together with the small quantity of quinine precipitated, is extracted, dried at 110°C., and weighed. A few drops of concentrated

hydrochloric acid are added, and the residue is again dried at 110°C. until free hydrochloric acid is expelled. This leaves quinine in the form of dihydrochloride of quinine, which is acid to methyl red and may be titrated with 0.02 *N* alkali and strychnine obtained by difference. Results obtained were not so good as the results obtained by collaborators on the Simmonds method.

This method is only slightly shorter than the Simmonds method. Strychnine is obtained only by difference, and it is difficult to detect the end point in the titration of dihydrochloride of quinine; therefore, in this respect it is not so satisfactory as the Simmonds method.

#### DISCUSSION.

The comments of various collaborators indicate that the method is fairly satisfactory, the gravimetric and volumetric results on strychnine being exceptionally good. It is extremely difficult to titrate quinine, which undoubtedly accounts for low results by titration. The method works smoothly but requires at least three days. Some tests made by the associate referee indicate that satisfactory results can be obtained by boiling the solution to hasten the precipitation of strychnine by potassium ferrocyanide and filtering while still warm; this, of course, would materially shorten the time required for the analysis.

It is the opinion of the associate referee that the method gives as good results as might be expected by any other method.

#### RECOMMENDATION<sup>1</sup>.

It is recommended that the Simmonds method as modified be adopted as a tentative method.

### REPORT ON SILVER PROTEINATES.

By MORRIS L. HITCHCOCK<sup>2</sup> (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

A review of the methods for the determination of silver proteinates showed that considerable work has been performed by this association.

In 1921 three methods for the determination of total silver were studied by W. L. Mitchell<sup>3</sup>. In 1923 E. O. Eaton<sup>4</sup> studied several methods, including a determination for total silver, a qualitative test for ionized silver, and two quantitative methods for ionized silver. Last year Eaton<sup>5</sup> submitted and recommended for tentative adoption a quanti-

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 78.

<sup>2</sup> Presented by C. K. Glycart.

<sup>3</sup> *This Journal*, 1922, 5: 542.

<sup>4</sup> *Ibid.*, 1924, 8: 49.

<sup>5</sup> *Ibid.*, 1925, 8: 551.

tative method for total silver, also qualitative and quantitative methods for ionized silver. These methods were adopted.

Inspection of the results of analyses by the method for total silver and the method for ionized silver as submitted in last year's report showed that close agreement was obtained by the collaborators. In addition to the report, further study of the importance of the ionized silver content of silver proteinates was made by Eaton and submitted to the Referee on Drugs by letters as follows:

Supplementing my report, "Methods for the Examination of Silver Proteinates", I wish to state that at the suggestion of a manufacturer's agent I have examined a number of these samples for acidity and alkalinity. The method used consisted of dialyzing a one-gram sample and titrating an aliquot representing 0.5 of a gram of the sample. Phenolphthalein was used as an indicator. The following results were noted:

*Strong Type.*

All the samples, Nos. 1, 2, and 3, contained ionic silver and the reaction was slightly acid.

*Mild Type.*

SAMPLE NO.

- 1.—Ionic silver absent; alkalinity 0.16 per cent as sodium hydrate.
- 2.—Ionic silver absent; alkalinity 0.48 per cent as sodium hydrate.
- 3.—Ionic silver absent; alkalinity 0.16 per cent as sodium hydroxide.

The reaction of the dialyzed solution in relation to the ionic silver is what would be expected. While the first action of inorganic silver salts is irritating and the first three samples reported herewith contained inorganic silver, it is therefore assumed that the small dosage and the irritant action are due to the ionic silver present. The last three samples reported contain no ionic silver, and therefore they are not irritating from that standpoint. Nevertheless, it is conceivable that the alkalinity of these samples might account for any possible irritating action.

Further supplementing my report, "Methods for the Examination of Silver Proteinates", I wish to say that I have just been able to obtain a sample of colloidal silver salt. This is a new product just on the market, and it is claimed to have all the advantages of the strong protargin type without the irritating effects of that type, also all the advantages of the mild type. Analysis shows this sample to be quite different from any others recorded to date, inasmuch as it contains a high percentage of silver and considerable ionized silver, and its solution is alkaline. The analysis is as follows:

Total silver—18.6 per cent by weight;

Ionic silver—1.2 per cent by weight;

Alkalinity as sodium hydroxide—0.24 per cent by weight.

In view of the recent determinations for acidity and alkalinity, it is possible that further collaborative work should be done on this subject, in which case the recommendations I have made should be changed. Personally, I think that the method as proposed should be recommended as tentative. I do not think the alkalinity or acidity is a very important factor from a medicinal standpoint, but I do think the ionic silver is.

No collaborative work was done this year owing to the associate referee's period of illness and subsequent resignation from the Bureau of Chemistry, but the study was kindly continued and this report prepared by C. K. Glycart.

Correspondence was had with F. W. Heyl, of the Upjohn Co., Kalamazoo, Mich., and it was his opinion that the total silver content of these salts is probably of minor importance in their clinical usefulness. He suggested that Sollmann's test be given consideration. This method, an antiseptic valuation of the ionized silver, has been published<sup>1</sup>. The following statements are made by Pilcher and Sollmann: "The fermentative activity of yeast cells furnishes a convenient and sufficiently accurate measure of the concentration of silver ions in solutions of the so-called 'colloid and protein silver compounds' as well as pure silver salts. Evidently, therefore, the content of silver ions is not only responsible for the irritant action which has been universally ascribed to them, but it is also responsible for all or nearly all the antiseptic action on yeast. In so far as the anti-bacterial efficiency of silver compounds is due to silver ions, this would be faithfully reflected by the yeast method. It would be immaterial in principle whether the silver ion concentration is measured by means of bacteria or of yeast, or by a chemical or a physical method. This, of course, does not mean that the minimum antiseptic concentration is the same for bacteria as yeast, for the absolute susceptibility varies with each organism".

For the work this year Sollmann's method was studied after changes in details were made. The directions are as follows:

#### PREPARATION OF SAMPLE.

*Strong silver protein.*—Transfer a 1 gram sample to a 500 cc. volumetric flask and add water to mark. Shake well. 1 cc. contains 2 milligrams of sample.

*Mild silver protein.*—Transfer a 2 gram sample to a 100 cc. volumetric flask and add water to mark. One cc. contains 20 mg. of sample.

#### REAGENTS.

(a) *Silver standard.*—Dissolve 100 mg. of C. P. silver nitrate in chloride-free water in a 2 liter volumetric flask. Make up to the mark. One cc. contains 0.05 mg. of silver nitrate. One cc. contains 0.03 mg. of silver.

(b) *Yeast-sugar mixture.*—Triturate 8 grams of commercial compressed yeast with 10 per cent cane sugar solution, transfer to a 200 cc. volumetric flask, and make to volume with the sugar solution.

NOTE: The mixture should be freshly prepared.

#### DETERMINATION.

Place 10 cc. portions of the yeast-sugar mixture into each of a series of 15 test tubes (1.5 x 15 cm.). For the control test, add from a graduated pipet to the mixture in the first 5 tubes 4, 4.5, 5, 5.5, and 6 cc. of the silver standard. Add 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 cc. of the prepared sample to the other 10 tubes. Add water to the control

<sup>1</sup> Pilcher and Sollmann. *J. Lab. Clin. Med.*, 8: 301; 9: 256.

and sample tubes to make the volume of each to 20 cc. Shake well. Fill small test tubes (0.8 x 10 cm.) with the mixture from the large tubes and at once invert into the larger tubes, being careful to allow no bubbles of air to rise in the smaller tubes. Place the tubes in a bath at 38°C. for one hour.

Under the conditions of the test, gas collects in some of the small tubes while no gas forms in the others.

For comparison select the control tube and the sample tube that show not more than a small bubble of gas. If no gas forms, repeat the test with less sample. If gas collects in all the tubes, repeat with a prepared sample of greater concentration.

To calculate the percentage of ionic silver in the sample, let

$A$  = weight of silver in the selected control tube, and

$B$  = weight of sample in the selected sample tube.

Then

$$\frac{A}{B} \times 100 = \text{percentage of ionic silver by yeast.}$$

Three samples were analyzed by M. L. Hitchcock and C. K. Glycart, with the following results:

LABEL	IONIC SILVER BY YEAST per cent
<i>Sample No. 1</i>	
Argentum Proteinicum (Imported)....	1.95
<i>Sample No. 2</i>	
Argentum Nucleinicum (Imported)....	0.996
<i>Sample No. 3</i>	
Argyrol.....	0.08

From the results of the preliminary work on this method, it appears that it is of practical value in differentiating between mild and strong types of organic silver compounds. It would be of interest, however, to prepare a series of results by both Eaton's and this method.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the now tentative method for total silver be adopted as official.

(2) That the tentative qualitative and quantitative methods for ionized silver be adopted as official.

(3) That the method for ionized silver by yeast be further studied for the purpose of comparison with the now tentative method for ionized silver by dialysis.

(4) That next year's associate referee consider whether work should be continued on the method for the alkalinity and acidity as proposed by Eaton.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 78.



## REPORT ON NITROGLYCERIN.

By ALFRED W. HANSON<sup>1</sup> (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

Nitroglycerin is the tri-nitric ester of glycerol. Mono- and di-nitric esters are also possible. As dinitroglycerin may be formed if insufficient nitric acid is used in the manufacture of trinitroglycerin, it is important that the required amount of nitrate is present. Nitroglycerin is used in tablets, triturates, and pills containing from 0.001–0.1 grain and is usually mixed with lactose. Spirit of glonoin is a pharmacopeial product and contains 1 per cent of nitroglycerin in alcoholic solution.

Ordinary nitroglycerin, containing three nitric acid groups, is a heavy oily liquid. Pure nitroglycerin is colorless, but the commercial article is yellow. The specific gravity of pure nitroglycerin at 15.6°C. is 1.60. Its solidifying point varies as it can be supercooled, but its true solidifying point has been given as 12°C. It is almost insoluble in water. Dinitroglycerin is soluble to the extent of 8 per cent in water. Both forms are soluble in ethyl ether. Trinitroglycerin is soluble in ethyl and methyl alcohol, chloroform, acetone, glacial acetic acid, and ethyl acetate. It differs from most oily substances by its insolubility in petroleum ether. Its high specific gravity also distinguishes it from vegetable oils. If an ethereal solution containing small amounts of nitroglycerin, as obtained in the assay of tablets, is evaporated at room temperature, the nitroglycerin is left in the form of oily droplets. According to Hess<sup>2</sup> it can be completely evaporated by continuous exposure at a temperature of 70°C., and it can be distilled in vacuo below 100°C. According to Guttman<sup>2</sup> a temperature between 45° and 50° C. is the critical one for nitroglycerin. The associate referee has found that four samples of nitroglycerin weighing about 0.03 gram each lost only about 0.2 mg. per sample when kept in a calcium chloride desiccator at a room temperature of about 26°C. for 24 hours. A rather violent decomposition takes place if a strong alcoholic potash solution is added to nitroglycerin. The residue should be dissolved in alcohol before treatment with potash. Nitroglycerin gives the diphenylamine reaction for nitric acid. The fact that nitroglycerin is volatile with steam may be useful in some cases when interfering substances are to be eliminated. This separation may not be quantitative as a portion of the nitroglycerin may be hydrolyzed.

When pure, nitroglycerin can be kept indefinitely without decomposition. Berthelot<sup>2</sup> records the keeping of a specimen for 10 years and McRoberts<sup>2</sup>, one for nine years without any appearance of decomposition. The presence of moisture or of a trace of free acid may start

<sup>1</sup> Presented by C. K. Glycart.

<sup>2</sup> Thorpe. *Dictionary of Applied Chemistry*, 1912, vol. II, pp. 428–434.

decomposition; sunlight may also cause decomposition. When nitroglycerin decomposes at ordinary temperature  $\text{NO}_2$  and  $\text{CO}_2$  are evolved.

#### PRODUCTS OF DETONATION.

The products of detonation of nitroglycerin are stated to be those of complete combustion and the equation representing the change is given as  $2\text{C}_3\text{H}_5(\text{NO}_3)_3 = 6\text{CO}_2 + 5\text{H}_2\text{O} + 6\text{N} + \text{O}$ . Nobel<sup>1</sup> has calculated that one volume of nitroglycerin on explosion generates about 1200 volumes of gases calculated to normal temperature and pressure and that the heat generated expands the gases to nearly eight times this volume. The explosive force of nitroglycerin is thirteen times as great as an equal volume of gunpowder.

The decomposition of nitroglycerin by caustic potash was stated by Railton<sup>1</sup> to be  $\text{C}_3\text{H}_5(\text{NO}_3)_3 + 3\text{KOH} = \text{C}_3\text{H}_8\text{O}_3 + 3\text{KNO}_3$ , but this was not at all justified by the results of his experiments as only a small fraction of the nitroglycerin seems to have been decomposed. Hay states that by the action of alkalis on nitroglycerin, glycerol is not regenerated, but is oxidized at the moment of formation at the expense of the nitric acid, potassium nitrite being formed. He sums up the reaction thus:  $\text{C}_3\text{H}_5(\text{ONO}_2)_3 + 5\text{KOH} = \text{KNO}_3 + 2\text{KNO}_2 + \text{HCO}_2\text{K} + \text{CH}_3\text{CO}_2\text{K} + 3\text{H}_2\text{O}$ , stating that while the oxidation products of the glycerol may vary as regards their nature and proportions, the above equation expresses with approximate accuracy the course of the reaction.

Berthelot<sup>2</sup> gives the following reaction:  $\text{C}_3\text{H}_5(\text{NO}_3)_3 + 3\text{KOH} = \text{C}_3\text{H}_8\text{O}_3 + \text{H}_2\text{O} + 2\text{KNO}_3 + \text{KNO}_2$ , and states: "Nitroglycerin is acted on by potassium hydroxide in aqueous and in alcoholic solution; but, naturally, much more slowly by aqueous potash".

The associate referee added alcoholic potash to a small amount of nitroglycerin. Gases were evolved having the odor of oxides of nitrogen, and the violence of the reaction indicated that it was not a simple saponification. (Compare with equation No. 1.) When nitroglycerin is greatly diluted with alcohol or water, the reaction with potash takes place quietly and no evolution of gas is noticed.

#### PREPARATION OF SAMPLE.

Each sample was prepared by mixing 35 grams of milk sugar with 8 grams of a nitroglycerin mixture such as is used in the manufacture of tablets. The nitroglycerin mixture consisted of nitroglycerin and milk sugar and assayed 8.81 per cent nitroglycerin by the proposed method. Owing to the danger involved in handling pure nitroglycerin, it is diluted with approximately 10 parts of milk sugar before it leaves

<sup>1</sup> Thorpe. Dictionary of Applied Chemistry, 1912, vol II, pp. 428-434.

<sup>2</sup> *Comp. rend.*, 1900, 131: 519.

the powder plant. This product is again mixed with milk sugar by the tablet manufacturer in the preparation of the tablets. The sample sent to the collaborators should contain 1.64 per cent of nitroglycerin.

#### LIST OF COLLABORATORS.

1. E. O. Eaton, U. S. Food and Drug Inspection Station, San Francisco, Calif.
2. C. K. Glycart, U. S. Food and Drug Inspection Station, Chicago, Ill.
3. A. W. Hanson.

#### COLLABORATIVE WORK.

Two methods were submitted to the collaborators for comparative study. Method No. 1 was received from A. G. Murray, Bureau of Chemistry, Washington, D. C., and Method No. 2, referred to as the proposed method, was prepared by the associate referee. The methods are as follows:

##### *Method No. 1.*

Take a sufficient number of the tablets to yield about one grain of nitrogen. Extract the nitroglycerin with successive small portions of ether, evaporating the ether in vacuum without heat. As a precautionary measure, take up the extracted excipient in water and extract the solution (or suspension) with ether, which is evaporated in a separate beaker. When the ether has been reduced to a volume of 3-4 cc., pour the ethereal solution into an 800 cc. Kjeldahl flask containing 100 cc. of water. Rinse the beakers with a little additional ether. (If desired, the extractions may be permitted to go to dryness and the extracts weighed. This procedure forms a valuable check if the tablets contain no soluble material other than nitroglycerin.) Add to the contents of the Kjeldahl flask a mixture of 30 cc. of approximately normal (4 per cent) sodium hydroxide, 60 cc. of water, and 10 cc. of 5 per cent potassium permanganate followed by an additional 100 cc. of water. Mix the contents of the flask thoroughly and allow to stand 10 minutes or longer. Heat the flask on the steam bath for a short time to assure complete saponification and to volatilize most of the ether. After thoroughly cooling, add 2-3 grams of powdered Devarda alloy, insert a stopper carrying the scrubber, and connect the apparatus with a vertical condenser by means of a Kjeldahl connecting bulb. (We used a special type of receiver for the distillate, the bottom of which consists of a narrow cup into which the end of the condenser dips. This permits the escaping gases to travel through a considerable depth of acid without the necessity of using a large volume.)

Use 50 cc. of 0.02 *N* sulfuric acid to absorb the ammonia. Heat the flask with a low flame as long as hydrogen continues to be evolved. Increase the flame and distil over about two-thirds of the contents of the flask. Titrate the excess acid with 0.02 *N* sodium hydroxide.

Use methyl red as the indicator, but do not add until after the distillation has been completed, as it has been found that it is destroyed by the hydrogen evolved during the process. Run a blank, using the same quantities of reagents, and apply correction to the determination. (The back titration can be made very accurately if the shade regarded as natural for the blank is carefully matched in the other titrations.)

To obviate any error which might be caused by alkali from the glass, a connecting bulb, condenser, and receiver manufactured of Pyrex glass was used. Only the inner tube of the condenser need be Pyrex. The condenser used was of homemade construction with a Pyrex inner tube.

For a description of the scrubber referred to in the method, see "A Scrubber for Ammonia Distillates", by Murray<sup>1</sup>.

#### STUDY OF QUANTITATIVE METHODS.

A study was made of a number of quantitative methods for the determination of nitroglycerin in medicinal preparations such as tablets and alcoholic solutions. A modification of the Devarda method for nitrates was prepared, in which the nitric groups are reduced to ammonia and estimated by distillation. This procedure and apparatus can also be applied to the determination of small quantities of inorganic nitrates. A suitable apparatus for this distillation, which can be prepared by any chemist, was also devised.

Owing to the rapid evolution of hydrogen when the alkali is added to a solution containing Devarda alloy, a fine spray which may contain some fixed alkali is formed when heat is applied. It is necessary, therefore, to pass the gaseous distillate through a scrubber-trap before it enters the condenser. The scrubber-trap, other apparatus, and method are described as follows:

##### *Method No. 2.*

Modified Devarda method applied to quantitative determination of nitroglycerin and a distillation apparatus for same.

##### APPARATUS.

- (a) *Kjeldahl distillation flask*.—800 cc.
  - (b) *Condenser*.—Water-cooled. Length about 22 inches. Pyrex glass recommended. An ordinary glass condenser gives good results.
  - (c) *Connecting bulb*.—About 3 inches in diameter (Hopkin's style). This style has a long inlet tube with an opening on the side. The end of the inlet tube can readily be passed through a small rubber stopper.
  - (d) *Adapter tube*.—About  $\frac{1}{8}$  inch in diameter at the top and terminating in a narrow outlet tube.
  - (e) *Receiving flask*.—A 500 cc. Pyrex glass recommended.
  - (f) *Miscellaneous accessories*.—A No. 7 rubber stopper for the flask and a No. 3 or 4 (2 hole) rubber stopper for the scrubber-trap.
  - (g) *Scrubber-trap*.—Made out of an ordinary Pyrex glass test tube about 6 inches long and 1 inch in diameter having a small flange at the top. When this scrubber-trap is connected up, it should extend from about 1 inch below the stopper in the flask nearly to the lower end of the neck of the flask. All the distillate should pass through this scrubber-trap, and the gas should be washed out by coming in contact with water. In order that the tube may be placed in the neck of the flask, the hole in the flask stopper should be about  $\frac{1}{4}$  inch off center.
- A two-hole rubber stopper is placed in the test tube. Through one of these holes a narrow glass inlet tube is inserted and extended to within  $\frac{1}{4}$  inch of the bottom of the test tube. The inlet tube of the connecting bulb is passed through the other hole, so that the inlet on the side of the tube is slightly below the stopper of the test tube.

<sup>1</sup> *J. Am. Pharm. Assoc.*, 1924, 13: 423.

In order to provide for drainage 3 or 4 capillary openings are drilled through the upper portion of the curved bottom of the test tube. These holes may be drilled by heating the tube in a gas flame below the point at which the glass becomes sticky and then working a stout red hot platinum wire through it, using a twirling motion. These openings should be high enough to permit some water to be retained in the tube during the distillation.

The inlet tube is made out of a narrow glass tube which has first been bent at right angles and one branch filed off close to the tube, thus giving it a very short side entrance.

This short bend is placed against the top of the stopper so that the tube will be held securely in place.

In order to provide increased scrubbing surface as well as to aid in the reducing action, three closely wound aluminum coils (No. 16 wire) about 2 inches long are placed one within the other in the bottom of the test tube, the outer coil fitting against the inside of the test tube.

After the tube has been prepared and the inlet tube of the connecting bulb has been inserted, a piece of thin, annealed iron wire, which has been twisted double, is looped under the flange of the test tube and wound around the inlet tube to the connecting bulb so that there will be no possibility of the test tube dropping off.

While the scrubber-trap described has been found to give satisfactory results, the scrubber referred to in Method No. 1, or any scrubber in which all the distillate is thoroughly washed with water before it leaves the distilling flask, may be used.

#### REAGENTS.

- (a) *Ethyl ether*.—Anhydrous.
- (b) *Devarda alloy*.
- (c) *Aluminum wire*.—About No. 16 gage.
- (d) *Alcoholic potassium hydroxide*.—Dissolve 15 grams of potassium hydroxide in 100 cc. of ethyl alcohol.
- (e) *Hydrochloric acid*.—0.02 *N* volumetric solution or 0.02 *N* sulfuric acid.
- (f) *Sodium hydroxide*.—0.02 *N* volumetric solution.
- (g) *Distilled water*.—Recently boiled and cooled. It should be free from ammonia.
- (h) *Methyl red indicator*.
- (i) *Ethyl alcohol*.—95 per cent.

#### PROCEDURE.

The method involves the following steps:

- (1) Extraction of the nitroglycerin from the sample with anhydrous ethyl ether.
- (2) Evaporation of the ether at room temperature after adding 10 cc. of ethyl alcohol.
- (3) Transfer of the alcoholic nitroglycerin solution to an 800 cc. Kjeldahl flask.
- (4) Preparation of the solution for distillation. Distilled water, 2 grams of Devarda alloy, 1½ inches of aluminum wire, and 15 cc. of 15 per cent alcoholic potassium hydroxide are added.
- (5) Refluxing for one hour at a low heat during which a rapid evolution of hydrogen takes place.
- (6) Distillation of the ammonia into 0.02 *N* hydrochloric acid or 0.02 *N* sulfuric acid.
- (7) Titration of excess of acid with 0.02 *N* sodium hydroxide, using methyl red indicator.

The procedure in more detail is described as follows:

Take a sufficient number of the tablets, which have previously been counted and weighed, to yield about ½ grain (0.0324 gram) of nitroglycerin. If the sample consists of powdered material, thoroughly mix before sampling. Place the sample in a 50 cc. beaker, add 10 cc. of anhydrous ether, and crush the tablets by means of a glass stirring

rod having a blunt end. Reduce the tablets to a fine powder so as to render the extraction complete. Decant the ether through a dry 7 cm. quantitative filter paper into a 250 cc. beaker in which 10 cc. of ethyl alcohol has been placed. Hold the filter paper in place in the funnel with the stirring rod and pour the ether down the rod. Make four more extractions in a similar manner. Dissolve the ether-insoluble residue in a small quantity of water, transfer it to a separatory funnel, and extract twice with 10 cc. of anhydrous ether. Filter the ether and combine the ether extractions. Remove most of the ether by evaporating the solution to about 10 cc. at room temperature by means of the air current from an electric fan.

Transfer the alcoholic solution containing the nitroglycerin to an 800 cc. Kjeldahl flask. Rinse the beaker with 10 cc. of ethyl alcohol and transfer it to the flask. Fill the beaker with distilled water and add the water to the flask. Dilute with distilled water to about 300 cc. Place the flask on an asbestos centered wire gauze. Add 2 grams of Devarda alloy (by means of a funnel) and about  $1\frac{1}{2}$  inches of heavy aluminum wire. Add 10–15 cc. of the alcoholic potassium hydroxide solution.

Immediately after adding the alkali blow a little distilled water into the scrubber-trap, insert the rubber stopper, and connect with an upright glass condenser, the lower end of which is fitted with a wide adapter tube dipping to the bottom of a 500 cc. Erlenmeyer flask in which about 25 cc. of 0.02 *N* acid and 10–15 cc. of water have been placed. Cool the condenser by running water.

Reflux the sample for one hour, using a short flame, the point of which is about  $\frac{1}{2}$  inch below the flask. Regulate the flame so that a rapid evolution of hydrogen takes place, but keep the top of the condenser bulb at about room temperature so that no liquid distils over. Increase the flame for 10–15 minutes until distillation commences and when active foaming ceases continue the distillation with a strong flame.

Whenever about an inch of water has accumulated in the trap, drain it by reducing the flame for a few seconds and then blowing on the outside of the flask. Continue the distillation until about 40 cc. remains in the flask. Lower the flame somewhat toward the end of the distillation to avoid cracking the flask. Remove the receiver containing the distillate, add sufficient methyl red indicator to make the solution red, and titrate back the excess of acid with 0.02 *N* sodium hydroxide. Calculate the nitroglycerin. Each cc. of 0.02 *N* acid neutralized by the ammonia is equivalent to 0.001514 gram of nitroglycerin.

Run a blank, using the same quantities of reagents and distilling in the same manner and make a deduction for same in the calculation. Care should be taken that the end points in the titrations are the same color.

#### *Collaborative results.*

	METHOD NO. 1	METHOD NO. 2 (PROPOSED)
	<i>per cent</i>	<i>per cent</i>
E. O. Eaton	1.71	1.71
	1.72	1.72
C. K. Glycart	1.635	1.654
	1.654	1.635
	1.540	1.635
	1.476	1.605
	4 gram sample	
A. W. Hanson	1.55	1.61
	1.53	1.63
	2 gram sample	
	1.60	1.61
	1.63	1.65

In order to test the accuracy of the proposed distillation method on alcoholic solutions of nitroglycerin and to determine whether the presence of milk sugar interferes with the determination, it was requested that the collaborators submit results on 2 grams of the sample and 25 cc. of alcohol, and also on aliquots of a clear alcoholic solution obtained by adding 95 per cent alcohol to 10 grams of the sample in a 200 cc. volumetric flask. The results are as follows:

*Sample direct + 25 cc. of alcohol.*

	<i>per cent</i>
E. O. Eaton.....	1.63 1.59
C. K. Glycart.....	1.587 1.565
A. W. Hanson .....	1.47 1.51 1.54

*Aliquots of clear alcoholic solution—Proposed Method.*

	<i>per cent</i>
E. O. Eaton.....	1.70 1.70
C. K. Glycart.....	1.69 1.76
A. W. Hanson.....	1.74 1.73 1.76

No allowance was made for the space occupied by the excipient in the preceding determinations. The associate referee obtained slightly lower results when a 10 gram sample was extracted with 95 per cent alcohol and the filtrate made up to 200 cc. The results were 1.67 and 1.66 per cent.

#### COMMENTS BY COLLABORATORS.

*Eaton:* The methods work satisfactorily.

*C. K. Glycart:* The results by the proposed method are in agreement with the highest figures obtained by Method No. 1. The proposed method eliminates the use of oxidizing reagents, which is a decided advantage over Method No. 1 since time is saved.

The scrubber-trap devised by Hanson is as efficient as the modified Davison apparatus.

The method of determining nitroglycerin in an aliquot from the clear alcoholic solution is rapid; however, the results obtained were higher. It is suggested that the volume occupied by the 10 grams of powder, which is insoluble in the alcohol, be considered, since less than 200 cc. of solution is obtained by these directions.

#### DISCUSSION OF RESULTS.

As nitroglycerin residues are slightly volatile at room temperature, the determination should be made as soon as possible. The addition of alcohol prevents the loss of nitroglycerin by volatilization.

The proposed method gives higher results than Method No. 1. It is possible to make a series of determinations in one day by this method. Method No. 1 required much more time, as it took about 24 hours to evaporate the ether with the vacuum pump available. The use of a vacuum may not be convenient in all laboratories, especially when the water supply is shut off at night. The distillation by both methods requires some attention to control the foaming. The first distillate may be redistilled, and close results should be obtained. The proposed method may be applied to the assay of spirit of glonoin (U. S. P.).

#### RECOMMENDATION<sup>1</sup>.

It is recommended that the proposed method for the determinations of nitroglycerin in tablets, powder, and alcoholic solutions be adopted as tentative.

### REPORT ON APOMORPHINE HYDROCHLORIDE.

By C. K. GLYCART (U. S. Food and Drug Inspection Station, Chicago, Ill.), *Associate Referee*.

Apomorphine hydrochloride is an artificial alkaloid prepared from morphine by the abstraction of one molecule of water<sup>2</sup>. It differs greatly from morphine in physiological action, being a powerful emetic. The fact that apomorphine hydrochloride can be administered hypodermically constitutes one of its chief advantages<sup>3</sup>. It is probably the most useful emetic and may be employed as such in the treatment of narcotic poisoning and in dislodging foreign bodies from the bronchi or esophagus<sup>4</sup>.

The usual methods of analysis of alkaloids are not suitable for apomorphine, because it readily oxidizes on exposure, turning a deep purple color.

In 1920, E. O. Eaton of the U. S. Food and Drug Inspection Station, San Francisco, Calif., in connection with the examination of investigational samples of apomorphine hydrochloride tablets, devised a method of analysis that permits titration of the alkaloid. Since the method has not been published, it was thought desirable to present it to this association for study. No claim for originality is made by the associate referee; the method is essentially as given by Eaton. A few details in manipulation with regard to testing for complete extraction have been added. Potassium bicarbonate reagent has been substituted for sodium bicarbonate, since its greater solubility in water facilitates its complete removal from the ether solution of the alkaloid<sup>5</sup>.

<sup>1</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9, 78.

<sup>2</sup> U. S. Pharmacopeia IX.

<sup>3</sup> May, *The Chemistry of Synthetic Drugs*, 1918, p. 111.

<sup>4</sup> National Standard Dispensatory, 1916, 3rd ed., p. 225.

<sup>5</sup> Lyons, *Practical Standardizing of Organic Drugs*, 1920, p. 189.



For the work this year a well-known manufacturer's product, labeled "Apomorphine Hydrochloride U. S. P. Crystals", was purchased on the market. The material was examined by the tests for purity given in the Pharmacopeia, and it was found to comply with the requirements.

Sample No. 1 represented powdered 1/10 grain tablets, which consisted of a mixture of 3 grams of apomorphine hydrochloride crystals and 6 grams of milk-sugar powder, U. S. P.

Sample No. 2 consisted of apomorphine hydrochloride crystals.

The samples and directions for analysis were sent to the following collaborators:

E. O. Eaton.

H. S. McCausland, The Abbott Laboratories, Chicago, Ill.

A. G. Murray, Bureau of Chemistry, Washington, D. C.

P. W. Morgan, U. S. Food and Drug Inspection Station, Chicago, Ill

Sample No. 1.—Use 0.4 gram sample for each determination.

Sample No. 2.—Use 150 mg. for determination.

#### REAGENTS.

(a) *C. P. potassium bicarbonate*.—10 per cent solution.

(b) *Washed ether*.—U. S. P. ether washed with water.

(c) *Sulfuric acid*.—0.02 *N* solution.

(d) *Sodium hydroxide*.—0.02 *N* solution.

(e) *Methyl red indicator*.

#### DETERMINATION.

(Undue exposure and delay in operations should be avoided, since apomorphine decomposes readily.)

Dissolve the sample in a small separatory funnel in 10 cc. of water. Add 5 cc. of 10 per cent solution of potassium bicarbonate and extract with 30 cc. of washed ether.

#### Collaborators' results.

APOMORPHINE HYDROCHLORIDE		
	Sample No. 1	Sample No. 2
	<i>per cent</i>	<i>per cent</i>
E. O. Eaton.....	29.46 29.50	96.7 99.2
H. S. McCausland....	30.36 30.10	97.2 98.2
P. W. Morgan.....	30.09 29.77	95.79 98.42
A. G. Murray.....	(a) 33.6 (b) 33.8 (c) 34.7* (d) 34.8*	(e) 98.1* (f) 98.7*
C. K. Glycart....	32.8 33.4	98.6 97.9

\* See Murray's comment for method used.

Transfer the aqueous layer to a second separatory funnel and repeat the extraction with 25 and 20 cc. portions of ether. Wash the combined solvent with 5 cc. of water. Transfer the wash water to the main aqueous solution and extract with 15 cc. portions of ether until the alkaloid is completely removed. Test for complete extraction by repeating the operation, using a separate beaker. Wash the combined total ether solvent with 5 cc. portions of water until the last wash water is neutral to methyl red and one drop of 0.02 *N* acid. Add directly to the ether in the separatory funnel such a measured quantity of 0.02 *N* sulfuric acid as will insure excess. Shake for one minute to neutralize the alkaloid completely. Withdraw the acid layer to an Erlenmeyer flask. Wash the ether with four 5 cc. portions of water and add these washings to the flask. Test for complete extraction. Immediately titrate the combined acid and wash water with 0.02 *N* sodium hydroxide solution and methyl red indicator.

One cc. of 0.02 *N* acid = 6.25 mg. of apomorphine hydrochloride.  $\frac{1}{2}$  H<sub>2</sub>O.

### COMMENTS.

*H. S. McCausland:* I find the end point, using methyl red, very unsatisfactory.

*P. W. Morgan:* I encountered difficulty in the determination of the true end point since the solution has a color which deepens as the alkaline titration proceeds.

*E. O. Eaton:* Both of these samples were grayish green and gave greenish solutions. Part of No. 2 was lost, therefore further work was not done on the sample.

*A. G. Murray:* I have found it practically impossible to "wash the combined total ether solvent with 5 cc. portions of water until the last wash water is neutral to methyl red and one drop 0.02 *N* acid". I believe the water extracts small quantities of alkaloid from the ether. The results identified as (a) and (b) were obtained by attempting to follow this procedure, 10 or 12 washings being made.

Results (c) and (d) were obtained by limiting the washings and taking the precaution of extracting the wash water with an additional 5 cc. of ether, which itself is finally washed and added to the main ether solution. Appreciably higher results were thus obtained, and I believe these figures represent the amount of alkaloid actually present. Results (e) and (f) also were obtained by this procedure.

The quantity of potassium bicarbonate solution specified seems unnecessarily excessive; 1 cc. would seem to be ample. This is the quantity used in all the determinations with the exception of (a), in which 5 cc. was used as directed. I do not believe that there is any difference in the effects of sodium bicarbonate and potassium bicarbonate. Results (a), (d), and (e) were obtained by the use of the potassium salts; results (b), (c) and (f) were obtained by using the sodium salt.

The quantities of ether specified also seem excessive; the alkaloid is apparently readily extracted by much smaller quantities of ether. Result (a) was obtained with the quantity of ether specified in your directions. In the other results 20 cc. of ether was used in the first extraction and 3 portions of 5 cc. each for the washing process. This, together with the additional 5 cc. used to wash the wash waters, makes a total of 40 cc. of ether for the entire procedure.

### DISCUSSION.

With regard to the lack of agreement in the results obtained on Sample No. 1, it seems that the variation is due to the details of the method rather than to decomposition of the different samples sent to the collaborators.

In the preparation of the material used for the sample, precaution was taken to prevent oxidation. The milk sugar and apomorphine hydro-

chloride were mixed with the aid of glass balls in a covered can. The sifting was made as rapidly as possible.

The results obtained on Sample No. 2 are fair. The average is below the theoretical. Considering the nature of the product, a control of 100 per cent was not expected.

With the view to greater accuracy the following items should be given consideration in the further study of the method:

- (1) Preparation of pure materials for samples.
- (2) The method to include specific directions for transfer of the ether extraction through the stem of the separatory funnel.
- (3) The effect of a large excess of 0.02 *N* acid on the end point.
- (4) The modifications by Murray with regard to the reduction in the quantity of potassium bicarbonate reagent and ether solvent, and also to the extraction of the wash water with ether.
- (5) Selection of the most suitable solvent.

#### RECOMMENDATION<sup>1</sup>.

It is recommended that the method be studied further.

#### REPORT ON SANTONIN.

By S. PALKIN (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

Santonin sells for about \$14 per ounce, or approximately \$200 a pound, and the crude drug, *Artemisia cina* Berg, or santonica, from which santonin is derived, costs from \$2-\$4 a pound. With the santonin content varying all the way from nothing to 3 per cent and with all the possibilities for substitution by substandard products, the need for a reliable and accurate method for the valuation of this drug is obvious.

From a chemical standpoint, santonin is an interesting compound. It is a stable lactone, but it can be converted readily to the acid form—santoninic acid—by treatment with alkali, and the free acid can be liberated and extracted as such. It reverts to the lactone form on contact with strong acid and when heated. In its natural state, in santonica, it is associated with resin acids, ethereal oils, etc., from which chemical separation, in the main, is not difficult. It is also associated with more neutral bodies, resinous or waxy in character and almost colorless, which are so like santonin in behavior that quantitative separation by the various chemical means ordinarily employed is virtually impossible.

The final purification of santonin by every method hitherto proposed depends on a crystallization from a hydroalcoholic solution, entailing a loss of santonin for which a correction must be applied.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 79.

## REVIEW OF LITERATURE.

A comprehensive, up-to-date literature review of this subject is available; in fact, it is much more than a literature review. Eder and Schneider<sup>1</sup> published a series of articles this year in which they made a systematic experimental study of practically all the methods for the assay of santonin that have been proposed; they also presented a method of their own embodying a few modifications of existing methods. No discussion of the details and relative merits of the various methods proposed is necessary in this report, as these points are thoroughly treated in Eder and Schneider's paper, but the following summary of the principles involved and of the details in which they differ essentially may be helpful:

(1) Practically all employ some organic solvent such as chloroform, benzol, ether, etc., for the first crude extract of the active principle. Some use a hot solvent—others, cold. One exception is noted in which aqueous alkali is used.

(2) All depend on the conversion of the santonin to a water-soluble salt of santoninic acid employing alkali, barium, or calcium hydroxide.

(3) Practically all specify the precipitation of the major portion of impurities—principally acid resins—in the form of barium, calcium, zinc or lead salts, one exception being noted, the method of Eder and Schneider, in which the extracted residue is boiled in a water-alcohol solution directly.

(4) All employ (with the Eder and Schneider exception) chloroform or other solvent for the extraction of santonin liberated from the acidified, partly purified filtrate.

(5) Some make use of a decolorizing material like kaolin or charcoal to remove colored contaminants.

(6) One is a titrimetric method, depending on alkali consumption of the santoninic acid after resin acids have been neutralized.

(7) Two physical-chemical methods, one embodying the use of a refractometer and the other, a polarimeter, subject the chloroformic extract to a washing with sodium carbonate solution to remove additional acid resin impurities and to make available a residue sufficiently free from color to permit the reading of its solution.

(8) With the exception of the physical, all the methods require a final purification by crystallization from hydroalcoholic solution, and the application of a correction factor for the santonin lost in the filtrate.

To the different methods that have been proposed may be added the numerous attempts described by Eder and Schneider to utilize the various known principles of analytical chemistry for the separation of the

<sup>1</sup> *Schweiz. Apoth. Ztg.*, 1925, 63: 29, 405, 30: 421; 31: 433; 32: 453

active principle from the associated contaminants. As finally concluded by these authors, none of the methods proved to be useful for a complete quantitative separation of santonin from the contaminating substances.

#### STUDY OF AVAILABLE METHODS.

This year the associate referee did not undertake a collaborative program, but confined himself to a study of available methods and to the development of some accurate quantitative method which would afford a complete separation from contaminants, would not involve crystallization from a solvent in which partial loss of this compound occurs, and which would not require a correction factor.

As may be known, the modification of the Katz-Fromme method is now tentatively official<sup>1</sup>, and unless some radical improvements, either for accuracy or rapidity, are afforded by a change, there would be no advantage in abandoning it.

A method developed by the associate referee offers promise of effecting a quantitative separation of santonin from its contaminating impurities without the application of a correction factor. As it is intended to publish the work when completed, only the principles involved and several assays of *santonica* to illustrate the application of the method will be given in this report. The associate referee will request the Referee on Drugs to appoint a new associate on santonin for the coming year in order that there may be less tendency for bias in the collaborative study of the various methods than might be the case if the writer were the referee.

#### METHOD.

One of the important facts brought out by Eder and Schneiter is that santonin can be readily extracted in the cold with solvents such as benzol and chloroform, and that the use of a continuous extraction apparatus such as the Soxhlet is unnecessary because the extract obtained in the cold is less concentrated in contaminants than when a hot solvent is used. In the method formulated by the associate referee acetone is employed as the extracting solvent, though the extraction can be made with benzol, chloroform, or other solvents, hot or cold.

Conversion of the santonin to salts of santonic acid is effected by treatment of the concentrated acetone extract (about 5 cc.) with alkali. The bulk of resin impurities is precipitated by the addition of a strong calcium chloride solution, and therefore the impurities are thrown out in a fine granular form and not collected in the form of a resinous mass, as is obtained by contact of barium hydroxide solution with chloroform in the Katz-Fromme method. The step involving saturation with carbon dioxide is also omitted.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 403.

The santonin is extracted from the acidified filtrate with chloroform and washed with alkali to remove the major portion of resin acids. Certain precautions must be taken to insure complete conversion to the santonin or lactone form, as free santoninic acid would be removed from the chloroform solution by shaking with alkali.

The residue from the chloroform extract is then purified from the weakly acid and neutral resins (and this is the principal novel feature) by conversion to the alkali salt and then to the calcium salt of santoninic acid under definite conditions and dilution largely with acetone. This leaves the calcium santoninate in the form of a white or nearly white precipitate and all the impurities in the acetone solution are retained. After filtration the santonin is liberated from the calcium salt with acid, extracted with chloroform, and determined gravimetrically in the usual way. The white product of a high degree of purity is obtained as shown by its melting point, 170°-171°C.

TABLE 1.

*Recovery of pure santonin by calcium chloride-acetone method.*

	SAMPLE TAKEN	RECOVERED
	gram	gram
No. 1 . . . . .	0.2000	0.199
No. 2 . . . . .	0.2000	0.2010
No. 3 . . . . .	0.1000	0.0995

TABLE 2.

*Determination of santonin in santonica.*

METHOD USED	SOLVENT	PURIFICATION (FINAL)	SANTONIN	COLOR OF RESIDUE	MELTING POINT
			<i>per cent</i>		°C.
1. A. O. A. C. . . . .	chloroform	15 per cent alcohol	3.01 2.86	very brown brownish	. . . . . .
2. New (defatted with petroleum ether)	benzol	calcium chloride acetone	2.85	white	171
3. New . . . . .	cold acetone	calcium chloride acetone	2.84	white	170
4. New . . . . .	cold benzol	calcium chloride acetone	2.81	white	. . .
5. A. O. A. C. modified*	chloroform	15 per cent alcohol	2.74	white	. . .

\* Chloroform extract washed with alkali, etc.

## RECOMMENDATION.

It is recommended that a collaborative program be undertaken to test two or three of the methods described.

No report on ether was given, as no associate referee had been appointed.

## REPORT ON BIOASSAY OF DRUGS.

By E. W. SCHWARTZE (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

No particular project was undertaken in this field for the following reasons: (1) Few members of the association could undertake the analytical work; (2) The United States Pharmacopeia X has not become official. The bioassay methods which the Pharmacopeia recommends and which were adopted after considerable study conducted similarly to that of this association are deemed sufficient for the present. It is proposed to take up U. S. P. methods for corroborative study just as soon as sufficient demand appears. However, under the present circumstances this is not anticipated—at least not until the methods have had an official trial.

It is recommended for next year that the method for the analysis of gossypol in cottonseed kernels be studied. While this is primarily a chemical method, it is not suitable in some cases. Bioassay of cottonseed meal is the only method usable under all conditions.

It is also proposed to take up the cat-eye method for standardization of mydriatics and also a study of the bioassay method for the active principle in thyroid products.

## CONTRIBUTED PAPERS.

## DETECTION AND DETERMINATION OF LACTIC ACID IN THE PRESENCE OF OTHER ORGANIC ACIDS.

By E. K. NELSON (Bureau of Chemistry, U. S. Department of Agriculture, Washington, D. C.).

The determination of lactic acid has been the subject of research by R. Kunz<sup>1</sup> and by Phelps and Palmer<sup>2</sup>.

The Kunz method is relatively simple, but it does not afford a satisfactory characterization of the acid. The method of Phelps and Palmer, depending, as it does, on the esterification of the acids and their subsequent separation by a special process of distillation, is altogether too complicated for the average analyst. However, Phelps and Palmer have found a satisfactory means for the identification of lactic acid, as their final product, quinine lactate, crystallizes well, has a definite melting point, and is easily prepared.

The development of a method utilizing the advantages of the Kunz and Phelps-Palmer methods was undertaken. The procedure evolved was subjected to a number of critical tests, and the results seem to justify a belief in its value.

## METHOD.

## DETECTION AND DETERMINATION.

For the estimation of lactic acid in fruit products, such as jams, jellies, or preserves, or in vegetable products (which should be comminuted) dilute 100 grams of the sample with 100 cc. of warm water, add 200 cc. of alcohol, and stir the mixture well. Separate the solids, including the precipitated pectin from the liquid by straining through a linen cloth, and evaporate the filtrate to 50 cc. In the case of medicinal preparations, filter a suitable quantity; if necessary, dealcoholize by evaporation, and bring to a volume of about 50 cc. In all cases the sample should be practically free from alcohol and volatile substances.

Acidify the solution with sulfuric acid in moderate excess and extract for 20 hours with ether in a Dunbar<sup>3</sup> lactic acid extractor, or, preferably, in a Palkin<sup>4</sup> extractor designed for extracting liquids with ether. Add 30 cc. of water to the extract, evaporate the ether, and transfer the solution to a separatory funnel. Shake out with chloroform, using five 10 cc. portions, to remove the benzoic acid. Reject the chloroform and subject the aqueous solution to steam distillation in order to remove the volatile acids, keeping its volume at about 30–50 cc., until 100 cc. of the distillate requires only 0.2 cc. of  $N/2$  sodium hydroxide to neutralize it.

Transfer the solution in the distilling flask to a beaker and add powdered barium hydroxide until alkaline to phenolphthalein and evaporate on the steam bath to a volume of 20 cc. If the alkaline reaction disappears, add more barium hydroxide.

<sup>1</sup> *Z. Nahr. Genussm.*, 1901, 4: 673.

<sup>2</sup> *J. Am. Chem. Soc.*, 1917, 39: 136.

<sup>3</sup> *J. Ind. Eng. Chem.*, 1911, 3: 930.

<sup>4</sup> *Ibid.*, 1925, 17: 612 (apparatus B)



Then pass carbon dioxide through the solution until neutral. Transfer the cooled solution to a 100 cc. cylinder, add 67 cc. of alcohol, and make up to volume with water. Shake well, filter, and wash the precipitate with a mixture of 2 parts of alcohol with 1 part of water. Evaporate the filtrate and washings to dryness; take up with 10 cc. of dilute alcohol, 2 : 1; filter through a small filter; and wash with a small quantity of alcohol, 2 : 1. This is done to remove the small quantities of barium citrate, malate, and tartrate which dissolve in 100 cc. of 67 per cent alcohol.

Evaporate the filtrate to dryness; dissolve in cold water; and, if necessary, filter again from alcohol-soluble organic material, which may appear at this point. Add an excess of a hot solution of quinine sulfate to the solution (0.5–1.0 gram of the sulfate should assure a liberal excess in most cases), cool as quickly as possible, and filter off the precipitated barium sulfate with any excess of quinine sulfate which may crystallize out. Wash with water and finally with alcohol in order to remove quinine sulfate, ignite, and weigh as barium sulfate.

Multiply the weight of barium sulfate by 0.7711 to obtain the corresponding weight of lactic acid.

#### IDENTIFICATION.

For identification, evaporate the filtrate which contains the quinine lactate and the excess of quinine sulfate to dryness in a vacuum distillation outfit on a water bath. Wash the dry residue once with carbon tetrachloride, decanting the solvent and removing the last traces with a current of air.

Add 25 cc. of dry alcohol-free chloroform. (Quinine lactate dissolves and quinine sulfate remains mostly undissolved.) Filter and evaporate the chloroform. Dissolve the residue in 10–20 cc. of hot absolute ethyl acetate (free from alcohol and water) and cool. Stir well to start crystallization and, if necessary, seed with a minute particle of pure quinine lactate. Recrystallize from absolute ethyl acetate or benzene, separate, and dry the crystals. Determine the melting point alone and admixed with pure quinine lactate. The melting point of quinine lactate, with some darkening and decomposition, is 165.5°C.

ACIDS TAKEN (DISSOLVED IN 100 CC. OF WATER)						LACTIC ACID FOUND		MELTING POINT OF QUININE LACTATE
Citric	Tartaric	Malic	Acetic	Benzoin	Lactic	gram	per cent	°C.
gram	gram	gram	gram	gram	gram			
1	1	1	1	0	1.0194	0.958	94.0	165–166
1	1	1	0	0	0.1125	0.1084	96	164–165
1	1	1	1	0	0.0412	0.0260	63	165–166
1	1	1	0.5	0.2	0.0331	0.0310	93.6	m. p. low but mixture gives higher m. p.
1	1	1	0.5	0.2	0.0494	0.0482	97.7	
1	1	1	0	0.2	0.0756	0.0624	82.5	164
1	1	1	1	0.2	0.1075	0.0929	86.4	157–160 mixed m. p. 161–164

A blackberry jam containing 47.5 per cent of fruit, 47.5 per cent of sugar, and 5 per cent of Douglas pectin was found to contain 0.067 per cent of lactic acid. The quinine lactate melted at 163°–165°C. The lactic acid comes from the Douglas pectin.

A similar jam, but containing 10 per cent of Douglas pectin, was found to contain 0.168 per cent of lactic acid. The quinine lactate melted at 163°–165°C.

In the numerous cases where the quantity of lactic acid is small and a low melting point may be found, the mixed melting point, or a crystallographic examination under the microscope, should settle the question of the presence or absence of lactic acid.

The preceding table gives the results of the determination of lactic acid in the presence of various organic acids.

#### CONCLUSION.

The detection and determination of lactic acid in the presence of malic, tartaric, citric, acetic, and benzoic acids and in fruit products have been successfully carried out by a modification of the Kunz and Phelps-Palmer methods. The characterization of the lactic acid as quinine lactate according to Phelps and Palmer is satisfactory.

#### DETECTION OF ADDED PEPPER SHELLS IN PEPPER.

By ERNEST R. SMITH, SAMUEL ALFEND, and LLOYD C. MITCHELL (U. S. Food and Drug Inspection Station, St. Louis, Mo.).

#### INTRODUCTION.

In the long history of food adulteration, pepper has probably received more attention from food analysts than any other product. As a result of the time and effort expended, however, it is now possible to detect the addition of any one of the hundred or more foreign substances which have been used to adulterate ground pepper. The microscope has proved to be a most useful instrument for this work. One form of adulteration has successfully defied the efforts of analysts at detection for many years—the addition of small quantities of pepper shells. It has been possible, heretofore, to detect the addition of 25, or perhaps even 20 per cent of added shells, but no smaller quantities. The two reasons for this limitation are the following:

*First*, pepper shell is a normal constituent of whole ground pepper. No definite information is available as to the amount of shell, or hull, in the whole pepper berry, and it is probable that the proportion is not constant in all berries of the same or of different varieties. The microscope is obviously of little use, and as there is no chemical constituent of pepper shell which is not found in whole pepper, a qualitative test for a shell constituent is of no value.

*Second*, the chemical constants of whole pepper vary considerably from sample to sample, so that no definite standards can be set which will make impossible the addition of pepper shells. A sample running higher in crude fiber and ash than the present standards is undoubtedly adulterated, but one running just within the limits cannot with certainty

be said to be free of added pepper shells, for it may have as much as 15 to 20 per cent of added shells and still come within the limits of the standards.

The determinations most used are total ash and crude fiber. Other determinations suggested as valuable by various analysts are acid-insoluble ash, starch, piperine, piperidine, volatile and non-volatile ether extract, alcohol extract, nitrogen, lead number, iodine number, pentosans, and ash constituents. Much of the work reported in the literature is of doubtful value because of the questionable authenticity of the samples, and particularly because of the varying or unknown methods of analyses. Even those analysts using the present official methods<sup>1</sup> may have obtained results not comparable with the results of others owing to variations in the preparation of the samples. This is especially noticeable in the crude fiber determination, in which the degree of fineness of the sample has a marked influence on the results obtained.

#### EXPERIMENTATION.

In attacking this problem, attention was centered on an attempt to determine whether there are any constituents whose values are constant for whole pepper, and much greater or much less for pepper shells.

#### SOURCE OF SAMPLES.

Samples of whole pepper representing the following varieties were obtained: 18 Lampong, 9 Alleppi, 5 Tellicherry, 2 Singapore, 4 white. There were also collected 4 samples of pepper shells and 3 of pepper siftings. These samples were obtained largely from importations at ports of entry—St. Louis, Chicago, and New York. Some samples of whole pepper and pepper shells were secured from spice manufacturers.

#### PREPARATION OF SAMPLES.

The samples were prepared for analysis by shaking 1–2 kilograms of the whole, unground pepper on a standard pepper sieve<sup>2</sup> to remove dust, sand, and small berries. Each sample was then handpicked to remove all other foreign materials. The most common impurities, the ones most likely to be included in commercial ground pepper, were found to be stones and clay particles about the size of the pepper berry, with which the pepper became contaminated during the drying process.

The content of the stems and siftings varied as follows:

VARIETY	STEMS AND SIFTINGS per cent		MATURE BERRIES per cent	
Lampong.....	0.7–5.0	Av. 2.7	0.8–6.0	Av. 4.0
Alleppi.....	0.1 or less		2.0–3.2	Av. 2.6
Tellicherry.....	0.2–0.5		3.0	
Singapore.....	0.7–1.7		2.3 and 3.2	

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 315

<sup>2</sup> Standard pepper sieve. Bryan Corcoran, Ltd., 31 Mark Lane, London, England.

The white peppers contained 10-12 per cent by weight of undecoricated pepper berries.

The stems, siftings, and other foreign matter from the black pepper were collected, and two samples were prepared from them by grinding to pass through a 40-mesh sieve. A sample, supposedly of ground pepper shells, but found on chemical analysis to have the same composition as the samples of stems and siftings, was classified with them. The cleaned pepper was then ground and screened through a 40-mesh sieve, and the coarse material was reground. This procedure was repeated until all the pepper passed through the sieve. It is important to sieve after each grinding to prevent part of the sample from becoming too fine. It is also necessary that all samples be of uniform fineness. Samples prepared as described showed no striation when placed in Mason jars and thoroughly shaken.

#### DETERMINATIONS.

The determinations selected as the most promising after a perusal of the literature were the following: (1) total ash, (2) water-soluble ash, (3) water-insoluble ash, (4) acid-insoluble ash, (5) alkalinity of water-soluble ash, (6) alkalinity of water-insoluble ash, (7) calcium oxide, (8) magnesium oxide, (9) phosphorus pentoxide, (10) manganomanganic oxide, (11) nitrogen, (12) alcohol extract, (13) d-glucose, (14) crude fiber, (15) non-volatile ether extract, and (16) moisture.

#### METHODS OF ANALYSIS.

The methods referred to in this paper are those found in *Methods of Analysis*, A. O. A. C., 1925, unless otherwise indicated.

#### MOISTURE.

The tentative method for moisture in spices, 2, p. 315, specifies that the sample be dried to constant weight at 110°C. Since apparatus for securing this temperature was not available, comparative tests were made by drying 2 gram samples as follows: (1) for 5 hours in an electric oven at 100°C.; (2) in a vacuum oven at 98°C. and 50 mm. pressure, in loosely covered dishes; and (3) in open dishes<sup>1</sup> in a vacuum oven at 98°C. and 50 mm. pressure. The average loss of weight on twelve samples, as determined in the vacuum oven in loosely covered dishes, was 10.15 per cent as compared with a loss of 9.26 per cent for the samples dried in the electric oven. Determinations on ten additional samples placed in the vacuum oven in loosely covered dishes yielded an average loss of weight of 9.45 per cent, whereas similar samples placed in the vacuum oven in open dishes gave 8.79 per cent. All determinations, therefore, were made by drying in a vacuum oven in loosely covered

<sup>1</sup> *This Journal*, 1924, 8: 76.

dishes. Inasmuch as no determinations of the volatile ether extract were made, the values termed "moisture" in this paper include the volatile ether extract and the moisture.

#### ASH AND RELATED DETERMINATIONS.

Total ash, water-soluble and water-insoluble ash, alkalinity of water-soluble and water-insoluble ash, and acid-insoluble ash were all determined by the following official methods: 3, 4, 5, p. 315, and 13, 14, p. 180. Four gram samples were used.

#### CALCIUM AND MAGNESIUM OXIDES.

The filtrate from the acid-insoluble ash was used for the determination of calcium and magnesium oxides. Calcium oxide was determined by the method given in 6, p. 41. A study of the collaborative work on which the present tentative method for magnesium<sup>1</sup> is based shows that the variation is too great for the purpose of this work. Magnesium oxide values on a sample of pepper analyzed by this method varied from 0.47–0.60 per cent. A "synthetic ash solution" containing iron, aluminium, manganese, calcium, magnesium, and phosphorus in the approximate proportions in which they are found in pepper, and having a theoretical magnesium oxide value of 0.850 per cent was prepared and analyzed by various procedures. The method finally adopted gave check results of 0.844, 0.850, and 0.853 per cent of magnesium oxide. Duplicate determinations of magnesium oxide in eight samples of pepper showed a maximum variation of 0.01 per cent, the average variation being 0.006 per cent. This method is as follows:

The combined filtrate and washings from the calcium determination, adjusted to a volume of about 100 cc., were treated with ammonium hydroxide solution, drop by drop, until the precipitate caused by the last drop failed to dissolve on stirring. This precipitate was dissolved by the addition of several drops of acetic acid solution, and 4–5 grams of ammonium acetate dissolved in water was added. A dilute solution of iron chloride was added, drop by drop, until a deep red color developed. Hot water was added to bring to a volume of 300 cc., and the solution was boiled until the basic iron acetate settled readily. The precipitate was filtered off on a large filter paper, without the use of suction, and washed with hot water containing a very little ammonium acetate. The solution was again treated with ammonium acetate and heated to boiling, and any additional precipitate was filtered off.

The manganese and remaining aluminium were removed by a modified Blum method<sup>2</sup>. The filtrate was adjusted to a volume of 150–200 cc.; it was then cooled and treated with a distinct excess of hydrochloric

<sup>1</sup> *This Journal*, 1921, 4: 392

<sup>2</sup> *J. Am. Chem. Soc.*, 1910, 38: 1282; *Ind. Eng. Chem.*, 1925, 17: 744.

acid, 5 drops of rosolic acid indicator (0.5 gram dissolved in a mixture of 50 cc. of 95 per cent alcohol and 50 cc. water), and 1 gram of solid ammonium persulfate. Ammonium hydroxide was added until a pink color just appeared, and the solution was heated to boiling and boiled for 1 minute. The precipitate was filtered off, washed moderately with a hot 2 per cent solution of ammonium chloride, redissolved in hot dilute hydrochloric acid, cooled, reprecipitated as before, and filtered. The combined filtrates and washings were treated with 25 cc. of concentrated nitric acid and evaporated to dryness, and the residue was heated on a hot plate to drive off ammonium salts. This residue was dissolved in water and the least possible quantity of dilute hydrochloric acid, and magnesium oxide was determined by double precipitation as phosphate, according to the procedure given in 7, p. 42, except that the addition of sodium citrate was omitted.

#### PHOSPHORUS PENTOXIDE.

One gram of the sample was moistened with 3-4 cc. of saturated calcium acetate solution, dried, and ashed at a low red heat. The ash was treated with concentrated nitric acid, and the solution was heated to boiling, diluted, and filtered. Phosphoric acid was determined in the filtrate by the official volumetric method, 10(a), p. 3.

#### MANGANOMANGANIC OXIDE.

Manganese was determined in the ash from 20 grams of the sample by the official method, 2, 8, pp. 39 and 42.

#### NON-VOLATILE ETHER EXTRACT.

The official method was used as given in 12, p. 117.

#### CRUDE FIBER.

The determination of crude fiber in ground pepper is influenced by the degree of fineness of the sample. As an illustration, it was found that crude fiber determined on a sample of commercial ground pepper, after the usual extraction of the fat with ether, was 15.56 per cent, whereas the same sample showed only 12.95 per cent of crude fiber when the material was ground to pass through a 40-mesh sieve. The determination was made on the residue obtained from the ether extraction by the tentative method, 17, p. 118.

#### D-GLUCOSE.

The d-glucose value was obtained by direct acid hydrolysis of a 2.5 gram sample of pepper without any previous treatment. The procedure otherwise is the same as that given in 21, p. 119, and 35, 36, pp. 190, 191. The weight of dextrose obtained is called the d-glucose value.

## NITROGEN.

Nitrogen was obtained by the official Kjeldahl-Gunning-Arnold method, 24, p. 8.

## ALCOHOL EXTRACT.

The alcohol extract was determined by the official method, 10, p. 316.

## DISCUSSION OF RESULTS.

The determinations that show a marked difference between whole pepper and pepper shells (see Table 1) are crude fiber, d-glucose, magnesium oxide, calcium oxide, total, soluble, and insoluble ash, alkalinity of ash, and manganese. Of these, only the magnesium oxide, crude fiber, and d-glucose are sufficiently uniform in the whole pepper to permit detection of the presence of small quantities (10–20 per cent) of added shells. These values, calculated to a dry basis, are given in Table 2. Whole pepper is seen to contain about half as much magnesium and crude fiber, and more than twice as much d-glucose as does pepper shell.

From Table 2 it is seen that  $\text{MgO} \times \text{crude fiber}$  ("A") and the  $\text{MgO}/\text{d-glucose}$  ratio ("B") are of definite value for approximating the amount of shells in pepper.

It will be noted that two samples of shells, Nos. 40 and 41, give values which indicate that they contain a considerable amount of endosperm. This condition is verified by the collection report of an inspector of the United States Bureau of Chemistry, who obtained these samples during the process of manufacture of a batch of "decorticated" pepper from whole black pepper. Samples Nos. 39 and 42, therefore, may be considered as more nearly representative of true pepper shells.

The several varieties of pepper differ slightly in composition, particularly in ash, crude fiber, d-glucose, and magnesium content. These differences may be explained by the smaller size of the pepper berries of the Lampong and Singapore varieties, with a corresponding larger proportion of shells. These two varieties also contain more dirt and empty shells.

Since Lampong pepper constitutes over 90 per cent of all pepper imported<sup>1</sup> into this country, these samples will be discussed first. On the basis of the crude fiber and d-glucose values alone, a sample falling just within the limits for Lampong pepper might contain 20 per cent of added shells. But from the magnesium oxide value and the values "A" and "B", it should be possible to detect the addition of 10 per cent of

<sup>1</sup> Spice Mill, 1925, 48: 2011.

shells even under the most unfavorable conditions. For example, if a Lampong pepper having the minimum values of 0.41 per cent for magnesium oxide, 5.2 for "A" and 7.4 for "B" (all on the dry basis) were adulterated with 10 per cent of shells, these values would be brought up to 0.45 for magnesium oxide, 7.0 for "A" and 9.7 for "B", as compared with the maximum values of 0.44, 6.4, and 8.9, respectively, for whole Lampong. With most lots of pepper, the addition of smaller quantities than 10 per cent of shells would bring these three values above the maxima.

Alleppi and Tellicherry peppers generally run slightly lower than Lampong in crude fiber and magnesium oxide and higher in d-glucose. It is obviously unsafe, therefore, in interpreting the results of an analysis, to base conclusions on the maximum-minimum figures for any one variety of pepper, unless the source of the product under examination is known.

The chemical composition of the samples classed as siftings can be predicted to a certain extent from their nature. They are characterized by fairly high crude fiber and magnesium oxide, very low d-glucose, and extremely high total and acid-insoluble ash. Failure to clean a lot of pepper properly, therefore, will tend to increase the crude fiber and magnesium oxide and decrease the d-glucose. The chief effect, however, will be to raise the values for total and acid-insoluble ash. When siftings instead of shells are added to ground pepper, as there is reason to believe has been done, the practice may be readily detected by the abnormally high ash content, without a corresponding abnormality in the other significant values.

#### SUMMARY AND CONCLUSIONS.

1. Values obtained from the analysis of 45 authentic samples of different varieties of whole pepper, pepper shells, and pepper siftings are given.
2. The most valuable criteria for detecting the addition of pepper shells to ground pepper are crude fiber, d-glucose, magnesium oxide, the ratio of magnesium oxide to d-glucose, and the product of magnesium oxide and crude fiber.
3. By the use of these values, and the authentic data given in this paper, the addition of small quantities of pepper shells may be detected. When the variety of pepper under examination is known, it seems possible to detect with certainty the addition of 10 per cent, or less, of shells.
4. The use of pepper siftings in place of pepper shells is readily detected by the abnormally high ash content without a marked change in the other significant values.



TABLE

*Results of analysis of authentic samples of whole*

SAMPLE NUMBER	MOISTURE	NON- VOLATILE ETHER EXTRACT	CRUDE FIBER	D-GLUCOSE	ALCOHOL EXTRACT	NITROGEN
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<b>LAMPONG</b>						
Maximum	12.38	10.74	13.70	51.32	12.26	2.28
Minimum	8.73	7.30	11.76	44.28	10.22	1.84
Average	10.06	9.29	12.50	47.20	11.20	2.09
<b>ALLEPPI</b>						
Maximum	10.86	10.46	13.02	51.76	14.34	2.22
Minimum	9.80	7.97	10.85	47.48	10.00	1.95
Average	10.13	8.95	11.66	50.36	11.15	2.08
<b>TELLICHERRY</b>						
Maximum	10.27	9.37	14.36	53.36	11.20	2.16
Minimum	8.20	7.25	11.56	51.20	9.60	1.97
Average	9.34	8.31	13.08	51.90	10.35	2.06
<b>SINGAPORE</b>						
33	11.23	9.50	13.83	49.00	9.54	2.28
34	11.00	9.35	13.92	49.04	9.94	2.15
<b>WHITE AND DECORTICATED</b>						
Maximum	11.65	9.70	4.86	76.56	9.36	2.02
Minimum	10.34	6.18	1.03	64.88	7.38	1.56
Average	11.05	7.79	3.64	68.64	8.50	1.89
<b>SHELLS</b>						
39	8.92	9.54	28.52	19.08	9.02	2.07
40	11.42	8.87	21.65	25.92	11.44	2.09
41	11.10	9.33	22.46	26.44	11.74	2.21
42	9.16	6.40	27.22	19.20	8.18	2.20
<b>SIFTINGS</b>						
43	7.07	4.20	17.04	11.92	5.82	2.47
44	6.22	3.93	19.49	13.84	5.50	1.79
45	8.08	5.09	22.58	20.68	6.24	2.14

1.

*pepper, pepper shells, and pepper siftings.*

Total	ASH					CaO	MgO	P <sub>2</sub> O <sub>5</sub>	Mn <sub>2</sub> O <sub>4</sub>
	Water-		Acid-Insoluble	Alkalinity of water cc. 0.1 N acid per 1 gram					
				Soluble	Insoluble				
	Soluble	Insoluble							
per cent	per cent	per cent	per cent			per cent	per cent	per cent	per cent
(18 samples)									
6.29	2.97	3.88	1.02	2.9	4.8	0.89	0.40	0.50	0.021
4.39	1.98	1.99	0.11	2.3	2.5	0.61	0.37	0.38	0.017
5.05	2.33	2.72	0.41	2.6	4.1	0.69	0.38	0.41	0.019*
(9 samples)									
5.83	3.61	2.66	0.36	3.0	3.9	0.67	0.39	0.46	0.018
4.18	2.50	1.70	0.02	2.4	2.3	0.53	0.34	0.40	0.016
4.74	2.88	1.86	0.11	2.6	3.3	0.60	0.37	0.44	0.017†
(5 samples)									
4.75	2.87	1.99	0.11	2.6	3.5	0.61	0.37	0.47	.....
4.41	2.54	1.71	0.07	2.4	2.4	0.54	0.35	0.42	.....
4.55	2.71	1.84	0.08	2.5	3.0	0.56	0.36	0.45	.....
(2 samples)									
4.17	2.32	1.85	0.16	2.4	3.4	0.59	0.38	0.45	.....
4.06	2.12	1.94	0.25	2.3	3.2	....	0.38	0.46	....
(4 samples)									
4.84	0.50	4.34	1.28	0.3	2.2	0.37	0.21	0.44	0.010
0.83	0.07	0.76	0.05	0.1	0.9	0.23	0.10	0.37	0.007
2.01	0.23	1.78	0.37	0.2	1.5	0.31	0.14	0.40	0.009†
(4 samples)									
10.84	4.09	6.75	2.40	4.8	7.9	1.09	0.72	0.38	0.032
7.19	4.49	2.70	0.30	5.1	5.3	0.87	0.57	0.40	0.027
7.32	4.42	2.90	0.30	5.2	5.5	0.89	0.59	0.31	0.021
10.89	3.46	7.43	2.22	4.1	7.6	1.08	0.72	0.33	0.040
(3 samples)									
37.16	0.88	36.28	20.45	0.7		0.37	....	0.37	.....
32.21	0.73	31.48	16.50	0.4		1.03	....	0.36	0.071
20.96	1.46	19.50	9.92	0.7		1.09	0.56	0.40	.....

\* Represents 3 samples.

† Represents 2 samples.

TABLE 2.

*Results of analysis of authentic samples of whole pepper, pepper shells, and pepper siftings computed to a dry basis.*

SAMPLE NUMBER	ON DRY BASIS			RATIOS	
	Crude Fiber	d-Glucose	MgO	MgO × CF	MgO × 1000
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		<i>d-Glucose</i>
LAMPONG (18 samples)					
Maximum	15.3	57.2	0.44	6.4	8.9
Minimum	13.1	49.5	0.41	5.2	7.4
Average	13.9	52.5	0.42	5.8	8.1
ALLEPPI (9 samples)					
Maximum	14.5	57.9	0.43	6.2	7.9
Minimum	12.1	52.4	0.38	4.6	6.7
Average	13.0	56.1	0.41	5.3	7.3
TELLICHERRY (5 samples)					
Maximum	15.8	59.2	0.41	6.2	7.2
Minimum	12.9	56.6	0.39	5.3	6.8
Average	14.4	57.5	0.40	5.8	7.0
SINGAPORE (2 samples)					
33	15.6	55.2	0.43	6.7	7.8
34	15.6	55.1	0.43	6.7	7.8
WHITE AND DECORTICATED (4 samples)					
Maximum	5.5	85.4	0.24	1.2	3.3
Minimum	1.2	73.2	0.11	0.1	1.4
Average	4.1	77.9	0.16	0.7	2.1
SHELLS (4 samples)					
39	31.6	21.2	0.80	25.3	37.8
40	24.4	29.3	0.64	15.6	21.9
41	25.3	29.7	0.64	16.2	21.5
42	30.0	21.1	0.79	23.7	37.4
SIFTINGS (3 samples)					
43	18.3	12.8			
44	20.8	14.8			
45	24.6	22.5	0.61	15.0	27.1

### METHOD FOR THE DETERMINATION OF ACIDITY OF HIGHLY COLORED FRUIT-TYPE PRODUCTS.

By C. H. BADGER and J. W. SALE<sup>1</sup> (Bureau of Chemistry, Washington, D. C.).

It is frequently impracticable to determine, by titration, the acidity of highly colored flavoring sirups, nectars, concentrates, and beverages because the artificial color masks the end point. While the use of phenolphthalein powder as an outside indicator is recommended for cer-

<sup>1</sup> Contribution from Water and Beverage Laboratory.

tain highly colored products<sup>1</sup>, it is a rather tedious method; moreover, certain combinations of permitted colors, such as are used in imitation grape, raspberry, strawberry, and other products are very similar to the red color of phenolphthalein, so that even this method is of doubtful value in many cases. Natural coloring matters may be removed by the use of lead acetate, but this reagent is not effective in removing the permitted coal tar colors.

#### A NEW METHOD.

A simple method for the determination of acidity when coal tar colors are present has been developed and successfully applied to commercial samples by the writers. The determination of the acidity of highly colored samples prepared in the laboratory indicates that the error by this method will not vary more than about 2 per cent, and it is frequently much less.

The principle of the method was suggested by the fact that acidulated solutions containing the permitted coal tar colors soluble in water, namely, ponceau 3 R, amaranth, orange I, naphthol yellow S, tartrazine, guinea green B, light green S F yellowish, and indigo disulfoacid can be rendered colorless or nearly so by placing pieces of woollen cloth in them and boiling. After such treatment the solutions can be easily titrated. Yellow A B and yellow O B are insoluble in water, and erythrosine precipitates on boiling.

#### PROCEDURE.

Place a sample containing 140–160 milligrams of acid and not more than 40 grams of sugar in a 500 cc. Erlenmeyer flask marked at the 100 cc. and 200 cc. levels. (The quantity of acid present in the sample can be estimated in a preliminary test by diluting a small portion of the sample enormously with distilled water made neutral to phenolphthalein and titrating, or by decolorizing a small portion of the sample with cloth and titrating as in the regular procedure. If the quantity of color is so great that it is impossible to decolorize a suitable sample, or if the sample contains only a small quantity of acid, a known quantity of hydrochloric acid may be added in order to make a suitable sample and apply a correction for the added acid.)

Dilute the sample with distilled water to the 200 cc. mark if it contains less than 200 cc. Add a piece of woollen cloth (nun's veiling) containing 15 square inches. Three by five inches is a convenient size, and the pieces may be cut by using an index card as a pattern. Heat to boiling and continue boiling until the liquid is level with the 100 cc. mark, using a glass stirring rod to turn the cloth occasionally and to hold it down in the liquid. Cool and pour the liquid into a 250 cc. beaker, draining slightly but without squeezing the cloth. Titrate the liquid in the beaker with 0.1 *N* sodium hydroxide, using 6–10 drops of a 0.5 per cent phenolphthalein solution as an indicator. If the decanted liquid contains considerable color, dilute with boiled distilled water before titrating. Pour 150 cc. of boiled distilled water on the cloth in the flask, rinsing down the sides of the flask; stir; and let stand about 4 minutes. Pour off this wash liquid into another 250 cc. beaker and titrate as before. Add another 150 cc. portion of boiled distilled water to the flask and after stirring let stand for about 3 minutes. Pour off this second

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 365.

*Acidity of highly colored samples prepared in laboratory.*

KIND AND QUANTITY OF COLOR PRESENT*	SUGAR PRESENT	KIND AND QUANTITY OF ACID ADDED†	ACID FOUND CORRECTED	ACID FOUND—PERCENTAGE OF THAT ADDED
<i>mg.</i>	<i>grams</i>	<i>mg.</i>	<i>mg</i>	
7a, 2i, 1o	26.5	138.4 C	136.4	98.6
7a, 3i	"	144.1 C	142.5	98.9
6a, 4i	"	149.9 C	146.8	97.9
5a, 5i	"	155.7 C	154.7	99.4
5a, 3i, 2o	"	138.4 C	138.2	99.9
5i, 5p	"	161.4 C	160.4	99.4
15a, 5i	"	145.4 C	144.3	99.2
15a, 5l	"	145.4 C	145.0	99.7
15a, 5g	"	145.4 C	144.7	99.5
15a, 5i	"	145.4 C	145.8	100.3
15a, 5l	"	145.4 C	146.1	100.5
15a, 5g	"	145.4 C	149.3	102.7
25a, 5i	"	139.6 C	138.2	99.0
20n, 20p	0	142.2 C	137.5	96.7
30a, 10l	"	139.2 C	135.0	97.0
30p, 10t	"	143.6 C	139.3	97.0
15a, 10p, 10t	"	146.6 C	142.2	97.0
10g, 5i, 25p	"	139.2 C	136.4	98.0
15a, 25p	"	143.6 C	141.8	98.7
10i, 30p	"	153.9 C	150.1	97.5
40a	"	142.2 C	138.6	97.5
10o, 30p	"	148.0 C	142.6	96.4
5i, 35p	"	145.1 C	140.8	97.0
5g, 35p	"	156.8 C	153.0	97.6
35a, 5i	"	158.3 C	154.4	97.5
5i, 5l, 30p	"	146.6 C	145.8	99.5
30a, 5i, 5o	"	146.6 C	142.9	97.5
5g, 5o, 30p	"	146.6 C	143.6	98.0
8a, 2i	26.5	160.2 T	161.9	101.1
8a, 2l	"	157.2 T	160.0	101.8
8a, 2g	"	154.2 T	156.2	101.3
2o, 8p	"	151.2 T	150.8	99.7
8p, 2t	"	145.1 T	147.7	101.8
8a, 2t	"	139.1 T	140.1	100.7
20n, 20p	0	146.7 T	147.0	100.2
30a, 10l	"	140.7 T	139.3	99.0
30p, 10t	"	149.7 T	150.4	100.5
15a, 10p, 10t	"	155.7 T	155.0	99.6
10g, 5i, 25p	"	140.7 T	141.2	100.4
15a, 25p	"	155.7 T	156.2	100.3
20a, 14i, 6p	"	161.7 T	161.2	99.7
30a, 10g	"	150.9 T	152.7	101.2
20a, 6l, 14n	"	140.7 T	140.8	100.1
30o, 10p	"	143.7 T	144.7	100.7
20a, 20o	"	146.7 T	144.3	98.4
40a	"	140.7 T	137.7	97.9
20a, 20i	"	158.7 T	158.1	99.6
25i, 15p	"	161.7 T	161.6	99.9
35o, 5p	"	140.7 T	139.7	99.3
20n, 20p	38	142.3 P	145.7	102.4
30a, 10i	"	140.2 P	146.7	104.6
20p, 20t	"	142.3 P	145.7	102.4
10i, 30p	"	140.2 P	145.7	103.9
20o, 20p	"	142.3 P	144.7	101.7
10i, 10o, 20p	"	150.7 P	150.4	99.8

\* a = amaranth; g = guinea green B; i = indigo disulfocid; l = light green S F yellowish; n = naphthol yellow S; o = orange I; p = ponceau 3 R; t = tartrazine.  
† C = citric acid; T = tartaric acid; P = phosphoric acid.

*Acidity of highly colored samples prepared in laboratory.—Continued.*

KIND AND QUANTITY OF COLOR PRESENT*	SUGAR PRESENT	KIND AND QUANTITY OF ACID ADDED†	ACID FOUND CORRECTED	ACID FOUND—PERCENTAGE OF THAT ADDED
<i>mg.</i>	<i>grams</i>	<i>mg.</i>	<i>mg.</i>	
2i, 8p	26.5	161.3 P	161.7	100.2
2l, 8p	"	157.6 P	160.5	101.8
2g, 8p	"	153.9 P	155.4	101.0
2i, 2l, 6p	"	150.3 P	152.4	101.4
2g, 2i, 6p	"	146.6 P	149.4	101.9
6a, 2g, 2i	"	139.3 P	140.7	101.0
10p	0	161.3 P	160.0	99.2
10a	"	153.9 P	151.7	98.6
10a, 1i	"	146.6 P	146.2	99.7
1i, 10p	"	139.3 P	138.6	99.5
10a, 1o	"	146.6 P	147.2	100.4
1o, 10p	"	146.6 P	145.9	99.5
20n, 20p	"	159.1 P	160.2	100.7
30a, 10l	"	150.7 P	153.7	102.0
30p, 10t	"	142.3 P	144.2	101.3
20a, 10p, 10t	"	140.2 P	140.9	100.5
10o, 30p	"	150.7 P	146.7	97.3
10a, 10i, 20p	"	159.0 P	161.0	101.3
10a, 5i, 25p	"	150.7 P	149.7	99.3
35a, 5o	"	142.3 P	140.4	98.7
5o, 30p, 5t	"	140.2 P	138.1	98.5
35a, 5l	"	150.7 P	150.4	99.8
35a, 5i	"	142.3 P	139.9	98.3
20n, 20p	"	142.3 P	144.7	101.7
30a, 10l	"	146.5 P	143.4	97.9
30p, 15t	"	150.7 P	152.7	101.3
10o, 30p	"	142.3 P	143.7	101.0
10a, 30p	"	140.2 P	142.4	101.6
20a, 10p, 10t	"	146.5 P	147.4	100.6

\* a = amaranth; g = guinea green B; i = indigo disulfoacid; l = light green S F yellowish; n = naphthol yellow S; o = orange I; p = ponceau 3 R; t = tartrazine.

† C = citric acid; T = tartaric acid; P = phosphoric acid.

wash into a 250 cc. beaker and titrate as before. The number of cc. of 0.1 *N* alkali used for the three titrations plus 2.20 (a correction value) represents the quantity of acid present in the sample.

#### DISCUSSION OF METHOD.

It was found impossible to remove all the acid from the cloth even after repeated washings. By following the directions carefully about 40 milligrams of color (dry basis) can be removed from a sample, although the quantity depends to a considerable extent on the kind of color and whether or not sugar is present. When sugar is present in the quantities usually found in imitation fruit sirups, about 20 milligrams of color can be removed.

Fortunately, those colors that interfere most seriously with the use of phenolphthalein as an indicator, namely, the red shades, dye readily and usually leave the liquid practically free from the interfering color. Light green S F yellowish and indigo disulfoacid do not dye quite so

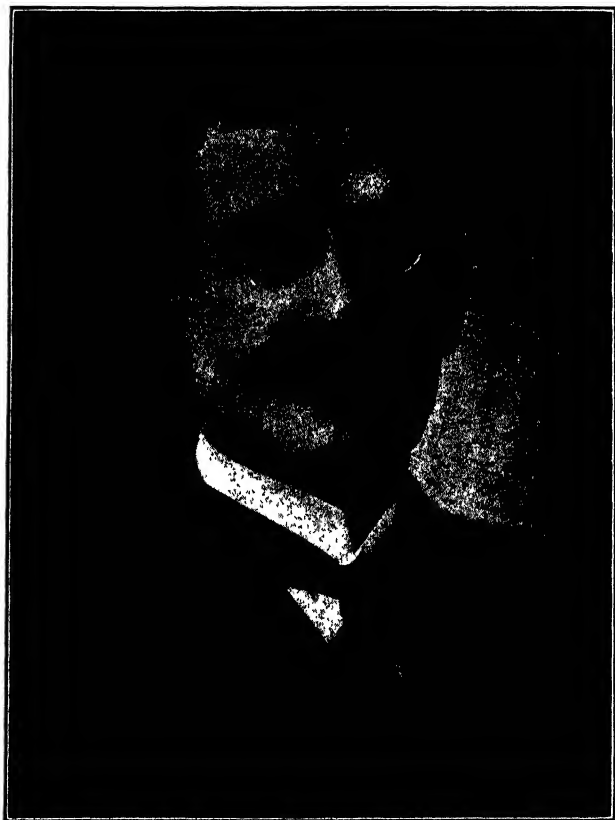
readily as the red and yellow shades. When appreciable quantities of guinea green B and orange I are present, a portion of these colors usually remains in the liquid after the boiling, but the end point can be detected without difficulty. Fairly accurate results can be obtained even if the sample contains more than 160 milligrams of acid, provided the other directions are closely followed.

#### DATA OBTAINED BY NEW METHOD.

In developing this method and testing its accuracy, many samples, some with sugar, simulating commercial products and containing in varying quantities combinations of the coal tar dyes and tartaric, citric, or phosphoric acid were prepared and analyzed. The data in the accompanying table were obtained by the method.







CHARLES LYNDALL PENNY, 1857—1925

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## CHARLES LYNDALL PENNY

Charles Lyndall Penny was born in Lewisburg, Pennsylvania, in 1857. He received his early education in the schools of his native town, and in 1879 he was graduated from Bucknell University with the B.A. degree. A few years later he received an M.A. degree from the same university, and later a degree of D.Sc. Following his graduation from the university, he taught chemistry in the Shippensburg Normal School until 1887, when he went abroad to spend a year in Bunsen's Laboratory in Heidelberg, Germany. Upon his return he went to Newark, Delaware upon the recommendation of Dr. Groff of Bucknell, and became chemist at the Delaware Agricultural Experiment Station. He held this position until 1907, when he resigned to become professor of agricultural chemistry at Pennsylvania State College. He remained in Pennsylvania but two years, for at the end of that time he was recalled to Delaware as professor of chemistry in the State University and as state chemist. He served as state chemist until that office was separated from the university in 1917, and as professor of chemistry and head of the department of chemistry in the University of Delaware until his death, October 20, 1925. He is survived by his wife, Mrs. Helena H. Penny.

As an agricultural chemist, Dr. Penny was well known for his research work on soils and fertilizers and on oil emulsions. His work on the relationship of nitrogen and the legumes is considered authoritative, and that on oil emulsions as spray materials is most valuable. In all his efforts he was exceedingly painstaking and careful, and was satisfied to give out conclusions only after repeated checks to show the accuracy of his results. His investigational work often led him to consult foreign journals, and he was proficient in French, German, Spanish, and Italian, as well as in Greek and Latin. He studied the higher mathematics as a diversion and delighted in difficult mathematical problems.

For a number of years Dr. Penny was a very active member of the Association of Official Agricultural Chemists, serving as a member of the Executive Committee in 1892 and again in 1903. He was elected vice-president for the year ending with the meeting in 1904, and president for the year ending with the meeting in 1905.

Dr. Penny possessed an unassuming disposition, and he could seldom be induced to talk about himself or his achievements. He had received the degree of D.Sc. from his alma mater a number of years before his associates in the University of Delaware learned of the fact through entirely outside sources. In his research he was thorough and painstaking. He was an ardent devotee of tennis and a skillful player. In his intercourse with students and fellow workers, he was thoughtful and exceedingly careful that injustice should never occur. His students could always find in him a friend and wise counsellor as well as an able teacher. Every acquaintance regarded Dr. Penny with deep respect and admiration and his friends, who were many, held him in sincere affection.

HERMAN H. HANSON.



## SECOND DAY.

### TUESDAY—MORNING SESSION.

#### REPORT ON CHEMICAL REAGENTS.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Referee*.

The report for the present year is concerned mainly with the activities of the Committee on Guaranteed Reagents of the American Chemical Society.

The first communication of the committee has been published<sup>1</sup>. It recommends specifications and tests for the following analytical reagents: Hydrochloric, nitric, oxalic, and sulfuric acids; ammonium hydroxide; ammonium oxalate; ammonium thiocyanate; barium chloride; iodine; potassium dichromate; potassium hydroxide; silver nitrate; sodium hydroxide; and sodium oxalate.

The recommended tests reflect the actual laboratory experiences of the five committee members, two of whom were interested from the viewpoints of reagent manufacturers, while the other three were concerned from the buyers' standpoint. These tests were reviewed and discussed by all the committee members through correspondence and in meetings held at stated intervals.

It is the desire of the committee that the new tests be fully tried out by chemists in all branches of the science and that comments and criticisms be freely addressed to the Chairman, W. D. Collins, Water Resources Branch, U. S. Geological Survey, Washington, D. C.

In the Bureau of Chemistry 122 reagent samples were examined, four of which were rejected.

Only one of these rejections, a sample of methanol, need be discussed. It was nearly 100 per cent in strength but gave a high residue on evaporation besides having a pronounced color and turbidity. This was evidently a carefully purified chemical that was afterwards carelessly handled.

#### RECOMMENDATION<sup>2</sup>.

It is recommended that observations on the quality of chemical reagents be continued and recorded as heretofore.

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<sup>1</sup> *Ind. Eng. Chem.*, 1925, 17: 756.

<sup>2</sup> For report of Sub-committee B and action of the association, see *This Journal*, 1926, 9: 75.

## REPORT ON EGGS AND EGG PRODUCTS.

By RAYMOND HERTWIG (Bureau of Chemistry, Washington, D. C.<sup>1</sup>),  
*Referee.*

The studies outlined at the last meeting of the association for accomplishment this year were seriously handicapped by the illness of M. L. Hitchcock and H. I. Macomber, associate referees, and the furlough from official duty of W. E. Kirby. No reports were submitted by these associates. Since Hitchcock's collaborative studies of the umpire vacuum method and a routine air-oven method for total solids in liquid and powdered dried eggs were considered most important this year, Associate Referee J. C. Palmer took them over and abandoned the work originally assigned to him. His cooperation is highly commended.

## COLLABORATIVE STUDY OF TOTAL SOLIDS METHODS.

Palmer submitted two methods for total solids in eggs to collaborative study—an umpire vacuum method which was adopted last year as official (first reading) and a routine air-oven method proposed by Hertwig and Bailey<sup>2</sup>. The latter is a more economical method for ordinary purposes than the umpire vacuum method and yields similar results.

Study of the collaborative results reported by Palmer and of the comments made by the various analysts regarding the sample of liquid egg furnished the collaborators, which was partially solidified by the addition of preservative and so made the drawing of representative portions somewhat questionable, and also regarding some slight variations in applying the methods, convinces the referee (1) that the umpire vacuum method is worthy of adoption as an official method (second reading) and (2) that the routine air-oven method gives results sufficiently close to those of the umpire method to entitle it to adoption as a tentative method. The routine method should be studied again collaboratively in preparation for its adoption as an official method, and the results obtained should be compared with those obtained by the umpire method. To circumvent the difficulties of submitting a satisfactory sample of liquid egg to a number of collaborators located at distant points, each collaborator should be requested to apply the two methods to a fresh egg sample obtained by himself. Subsamples of a dried egg, however, can be furnished each analyst as was done this year.

COLLECTION AND PREPARATION OF SAMPLE OF FLAKED  
DRIED EGG.

The tentative methods adopted at the 1924 meeting for the collection and preparation of sample of different types of eggs<sup>3</sup> did not include

<sup>1</sup> Present address: Hecker-H-O. Co., Inc., Buffalo, N.Y.

<sup>2</sup> *This Journal*, 1925, 8: 451.

<sup>3</sup> *Ibid.*, 599.

flaked dried eggs. It was recommended that consideration be given to this matter this year. The method for collecting a sample of flaked dried egg is similar to that for powdered dried eggs, but the sample must be ground in a mill before being analyzed. This step, however, is not difficult. The method is described in the final recommendations of this report.

FAT (ACID HYDROLYSIS METHOD), LIPOIDS, AND LIPOID  
PHOSPHORIC ACID ( $P_2O_5$ ).

The tentative methods for fat (acid hydrolysis method), lipoids, and lipid phosphoric acid ( $P_2O_5$ ) were adopted as tentative in 1924. The referee has found that these methods are proving satisfactory in the hands of many analysts. No adverse comments have been received. It is believed that these methods, as given in the referee's 1924 report<sup>1</sup>, should be made official.

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY  
40 PER CENT ALCOHOL.

The method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol with certain modifications was recommended for further study at the 1924 meeting. Some work was accomplished in this direction.

It was recommended by Palmer in his 1924 report<sup>2</sup> that a 1.2 per cent salt solution be used as an extractive for the proteins instead of water. He was inclined to believe that the soluble proteins passed into dilute salt solution more readily than into a water solution, although similar results are obtained by both extractives.

Since this method is used particularly for detecting added egg albumin in cereal products, it is desirable that the method applicable to both egg and cereal products be kept the same so far as is possible. Palmer and the referee, working independently, found that a 1.2 per cent salt solution extracts considerably more protein precipitable by 40 per cent alcohol from wheat flour than does water. Because it is the intended purpose of the method that only water-soluble proteins precipitable by 40 per cent alcohol and not salt-soluble proteins precipitable by 40 per cent alcohol be determined by the method in cereal-egg products, it is believed that water and not a salt solution should be the extractive in this method as applicable to eggs.

In the studies made by Palmer in 1924 difficulties were encountered in filtering the precipitated albumin from the mother liquid. He recommended, therefore, that the nitrogen in the precipitate be ascertained in an indirect manner by determining both the nitrogen in an aliquot

<sup>1</sup> *This Journal*, 1925, 8: 601.

<sup>2</sup> *Ibid.*, 615.

of the extract solution without the addition of alcohol and the nitrogen in the supernatant liquid of another aliquot after precipitation with alcohol and the calculation of the nitrogen in the alcohol precipitate by difference. Since indirect methods combine the errors of two determinations it was thought inadvisable to adopt such a procedure until direct determination was proved to be impracticable. Consequently, at the 1924 meeting the referee recommended the study of a mechanical separation other than by filtration of the precipitated albumin from the mother liquid. The referee has found that this is a practical procedure if the centrifuge is used, and he was able to determine readily in this manner the alcohol-precipitable protein in a sample of dried egg albumin, which is almost impossible by the original method.

It was found that centrifugalization of the mixture containing the precipitate in a suitable tube tapered at the bottom, furnishes a convenient and rapid method for the separation and washing of large quantities of the precipitated protein. A satisfactory centrifuge tube, capacity about 125 ml., diameter about 4 cm., and length about 15 cm., is readily made from a heavy glass tube. The bottom of the tube for a length of 6-8 cm. is tapered down to a diameter of about 1 cm. at the sealed end. Centrifugalization firmly packs the precipitated protein in the constricted end and permits the decantation of the supernatant liquid. To prevent breakage during centrifugalization, the tube should be cushioned in a rubber stopper that fits snugly in the trunnion cup.

#### MODIFICATION OF METHOD.

##### Directions for the proposed modification follow:

Transfer the mixture of alcohol, precipitate, and 0.2-0.5 gram of prepared ignited asbestos specified by the method to the centrifuge tube. Mix the asbestos and precipitate well and whirl at a high speed until they are packed firmly in the bottom of the tube. Decant the supernatant liquid through an asbestos mat in a Hirsh funnel, using a light suction. Add about 20 ml. of 40 per cent alcohol to the tube and mix well with the precipitate with the aid of a glass rod, rinsing off the rod with a little 40 per cent alcohol. Centrifugalize and again decant off the supernatant liquid through the same filter mat. Wash medium quantities of the precipitate twice and large quantities three times. Transfer the precipitate and asbestos to a Kjeldahl flask by means of a stream of hot water from a wash bottle, using a rubber policeman to remove any precipitate adhering to the tube. Add the filter mat to the Kjeldahl flask and proceed as directed by the method.

A 2 gram sample of dried egg albumin was shaken with 200 ml. of water and 50 ml. of the filtered solution was used for the precipitation. Results for the water-soluble protein-nitrogen precipitable by 40 per cent alcohol of the dried egg albumin as determined in the above manner were found to be 9.80 and 9.75 per cent.

This proposed method should be studied during the coming year with a view to the modification of the original method so as to include the

procedure of separation and washing of the alcohol precipitate free from the mother liquid. Attention should also be given to the water extraction of the sample to insure that all the water-soluble proteins are actually extracted.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the umpire vacuum method<sup>2</sup> for total solids in eggs as given in the referee's 1924 report be adopted as official (final action).

(2) That the routine air-oven method for total solids in eggs be adopted as tentative. This method has been published<sup>2</sup>.

(3) That the routine air-oven method for total solids in liquid and dried eggs be studied during the coming year. In this study it is suggested that each collaborator analyze a sample of fresh eggs obtained by himself and make comparison of results with those obtained by the umpire vacuum method.

(4) That the following method for collection and preparation of sample of flaked and drum dried eggs be adopted as tentative and be published with the methods for the collection and preparation of samples of liquid and powdered dried eggs:

(d) *Flaked and drum dried eggs:* Collect the sample as directed for powdered dried eggs. Report odor and appearance and examine for foreign material. Prepare albumin samples for analysis by grinding in a mill to pass entirely a 60-mesh sieve, and whole egg and yolk samples to pass entirely a 20-mesh sieve or as fine as is practicable. Keep in a hermetically sealed jar in a cool place.

(5) That the acid hydrolysis method for the determination of fat in eggs, as given in the referee's report for 1924<sup>3</sup>, be adopted as official (first reading).

(6) That the method for lipoids and lipid phosphoric acid ( $P_2O_5$ ) in eggs as given in the referee's report for 1924 be adopted as official (first action).

(7) That the method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol in eggs be further studied. It is suggested that these studies include the separation and washing of the alcohol precipitated protein freed from the mother liquid by the aid of centrifugalization as proposed in this report, and also the complete extraction of the water-soluble proteins from the sample.

(8) That the method for the determination of ash in eggs be studied during the coming year, particular consideration being given to the type of material of the ashing dish.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 82.

<sup>2</sup> *This Journal*, 1926, 9: 57.

<sup>3</sup> *Ibid.*, 1925, 8: 601



(9) That the method for organic and ammoniacal nitrogen in eggs given in the referee's report for 1924 be adopted as official (final action).

(10) That methods for unsaponifiable matter in eggs be studied during the coming year.

(11) That the parenthetical statement, "Report results as percentages of the original sample and of the total solids contained therein", be inserted in the next revision of *Methods of Analysis* immediately under the chapter heading "Eggs and Egg Products".

(12) That the study of methods for zinc in eggs be continued during the coming year.

(13) That study of the method for acid-soluble phosphoric acid in eggs be continued during the coming year. It is suggested that this study include consideration of (1) the addition of the picric acid to the extraction mixture at the end of the half-hour period of shaking instead of at the beginning; (2) the determination of phosphoric acid by the volumetric method instead of by the gravimetric method; (3) any other means of simplification of the method.

(14) That methods for the acidity of lipoids be studied during the coming year.

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*H. W. Redfield:* I wish to speak concerning the recommendation of the referee that the method for determining total solids in eggs by drying in vacuum at 98°–100°C., and at a pressure of not over 25 mm. be adopted as official, second reading, and also concerning the recommendation that the method for determining total solids in eggs by drying at atmospheric pressure at 112°–117°C., be adopted as tentative, first reading.

In 1917 the Bureau of Chemistry carried on an extensive investigation for the purpose of developing methods which could be employed in determining definitely whether liquid and frozen eggs had been prepared in whole or in part from inedible eggs. A large number of authentic samples were prepared in commercial egg-breaking plants by a group of experts who carefully examined all eggs used and so were able to characterize each sample definitely. These samples, of commercial size, were placed in cold storage for a month or so and then were simultaneously analyzed by five chemists and three bacteriologists. The chemical methods chosen for this purpose were the best known at that time. They included total solids, ether extract, ammonia nitrogen, reducing substances, and acidity of the fat. The total solids were determined by drying to constant weight at 55°C. in a vacuum oven at not less than 25 inches vacuum. The dried solids were extracted with ether for the determination of ether extract, and this ether extract was then used for the determination of acidity of the fat. The description of the samples,

methods of analysis, results of analysis, and a scheme for interpreting the results so as to differentiate positively between edible and inedible eggs were published as U. S. Department of Agriculture Bulletin 846.

In interpreting the results, the ammonia nitrogen and acidity of the fat are both compared with the ether extract, while the reducing substances are compared with the figure secured by subtracting the ether extract from the total solids. This figure approximates the true figure for solids-not-fat, and for purposes of brevity I will so designate it.

This scheme for reporting results has stood the test of time for over seven years with never a failure. It would, therefore, appear to be valuable. It has been used extensively, especially by the New York Station, where large shipments of imported frozen eggs are frequently offered for entry and where also there is heavy trading in the domestic product. It is not uncommon for single shipments of Chinese frozen eggs worth a million dollars or more to be entered at the port of New York.

The recommendation of the referee concerning the determination of total solids is, therefore, a matter of very grave concern to the New York Station of the Bureau of Chemistry. At my request Mr. Macomber of that Station conducted certain experiments for the purpose of determining whether the analytical figures for the authentic samples, as published in Bulletin 846, are applicable for comparative and interpretative purposes, if total solids are determined by either or both of the methods proposed by the referee, and if from such total solids, ether extract, and acidity of the fat are then determined. In this experiment two series of three samples each were prepared. In each series, a thoroughly mixed sample of fancy eggs, of second-grade eggs, and of inedible eggs were analyzed for total solids by the method given in Bulletin 846, by the proposed official method, and by the proposed tentative method. From the total solids, in each instance, the ether extract and the acidity of the fat were determined by the methods given in Bulletin 846.

Without burdening you with the analytical figures secured, it will suffice to say that in all cases the total solids by the 112°–117°C. method and by the 100°C. method were, respectively, 0.4 per cent and 0.2 per cent lower than by the 55°C. method. On the other hand, the ether extract from the solids dried at 112°–117°C. and at 100°C. averaged, respectively, 0.64 per cent and 0.69 per cent higher than from the solids dried at 55°C. In the matter of the acidity of the fat, results averaging, respectively, 0.08 per cent and 0.22 per cent higher were obtained from the solids dried at 112°–117°C. and at 100°C. than at 55°C., in the case of fancy eggs and second-grade eggs. The reverse was true in the case of inedible eggs, where lower results for acidity of the fat were obtained from the solids dried at 112°–117°C. and at 100°C. than from those dried at 55°C., the difference being, respectively, 0.31 and 0.92 cc. of 0.05 *N* sodium ethylate per gram of fat.

As the results for ether extract and solids-not-fat are used in interpreting the results for ammonia nitrogen and for reducing substances as well as for acidity of the fat, we are forced, with regret, to conclude that if total solids are determined in eggs by either of the methods proposed by the referee, then the method of interpretation given in Bulletin 846 is entirely inapplicable and the thousands of dollars spent in compiling that bulletin will have been wasted.

It would further appear from the analytical results already referred to, and from the fact that the ether extracts of the solids dried at 112°–117°C. and at 100°C. were very dark in color, that decomposition of the egg material by both of the proposed methods is indicated.

### REPORT ON METHODS FOR THE DETERMINATION OF TOTAL SOLIDS IN LIQUID EGGS AND POWDERED DRIED EGGS.

By J. C. PALMER (U. S. Food and Drug Inspection Station, San Francisco, Calif.<sup>1</sup>), *Associate Referee*.

M. L. Hitchcock of the U. S. Food and Drug Inspection Station, Chicago, was originally appointed to carry out collaborative work relating to the solids determinations of liquid and dried eggs, but owing to his illness the present associate referee was asked to complete the work already started.

Two samples were prepared and distributed to the collaborators, one of liquid egg and one of powdered dried whole egg.

The liquid egg sample was prepared as follows: 18 fresh eggs were shelled and well mixed by beating with a malted milk stirrer. Ten cc. of 40 per cent formaldehyde solution was added as a preservative, and the product was placed in 6 ounce bottles and hermetically sealed with paraffin.

The powdered dried whole egg, a commercial sample imported from China, was prepared by passing through a domestic flour sifter three times. The product was well mixed after each sifting and finally placed in 6 ounce bottles hermetically sealed with paraffin.

The samples were submitted to collaborators with the directions for the umpire vacuum and rapid routine methods as published previously<sup>2</sup>.

### COMMENTS BY COLLABORATORS.

*W. S. Arnold:* In December, 1921, we ran a series of tests in our vacuum oven (using a water pump which produced only 26 inches of vacuum) which indicated the procedure then adopted by our laboratory as a standard routine method. This method was as follows:

<sup>1</sup> Present address: Bureau of Chemistry, Washington, D. C.

<sup>2</sup> *This Journal*, 1925, 8: 630; 1926, 9: 56.

Weigh 5 grams of the dried yolk or egg into a 55 mm. aluminum dish and dry at 99°–100°C. in a vacuum of 26 inches, or more, for 2 hours. Leave the dish entirely uncovered while in the oven and do not dry the air used to release the vacuum.

This method gives very satisfactory results, and close checks can be obtained. It is rapid and easy and requires a minimum of apparatus. We found by comparison that the drying of the air used to break the vacuum affected the results so little as to be of no practical consequence.

*J. L. Heid:* I regret to say that the sample of liquid egg arrived in a condition unsuitable for analysis. It was partially coagulated and separated in such a manner as to make the obtaining of uniform samples impracticable. It is thought that formaldehyde might have been used as a preservative and that this, with the hot weather, resulted in the coagulation.

One accident occurred in the analysis of the dried egg sample. The vacuum was accidentally shut off at the end of the fourth hour and I did not discover it until the end of the fifth hour. I turned it on once more and ran the sample an additional hour and fifteen minutes.

The routine method gave me higher results, as was to be expected. It is susceptible to variations in atmospheric conditions, and the work was done on a day when the temperature was 32°C. and humidity close to 100°. It is believed that better results can be obtained, where samples must be shipped so far, by doing the work in the winter months.

*O. S. Keener:* Owing to the continuous use of the laboratory electric oven, in butter work at 100°C., the hot air oven was used instead. The temperature for most of the time was kept between 115° and 120°C., but at several brief intervals it ran as high as 125°C.

*L. H. Bailey:* The liquid egg was of a creamy consistency, but it could be mixed to a homogeneous mass.

The results are not in as close agreement as I hoped to obtain. The pressure in the vacuum oven was about 12–13 mm., and I think this may have resulted in a little greater loss in weight in the samples dried in the vacuum oven.

*R. L. Horst:* In the case of the liquid eggs I wish to state that this sample was partially coagulated when I opened it after keeping it in the ice box, but it was thoroughly mixed. I believe that most of the separated water was reincorporated with the solids and that the sample was as homogeneous as possible.

*R. S. Fleming:* In preparing the liquid egg, we removed the sample from the bottle and ground it for some time in a mortar. There might have been a slight loss of moisture during this grinding, but whether it was enough to affect the results or not, I cannot say. Portions of liquid egg after drying out appeared rather lumpy, and it occurred to the writer that it might be a good thing to add a little water to the samples after they are weighed up but before they are dried. One series of tests was made this way as indicated in the report. Judging by the results, there is no particular advantage in doing this.

It was thought that since the liquid egg could not be smoothly distributed over the bottom of the dish, there might be some moisture retained in lumps which had "crusted over". So water was added to two weighed samples and they were then thoroughly mixed with a glass rod, after which the particles of egg adhering to the rod were washed into the dish by means of a wash bottle. The results of this procedure are as follows:

*Results of collaborators on solids*

ANALYST	DRIED WHOLE EGG		LIQUID EGGS	
	Umpire Vacuum	Rapid Routine	Umpire Vacuum	Rapid Routine
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
W. S. Arnold The International Co. Baltimore, Md.	94.35 94.28	93.89 94.02	26.23 26.31	25.98 26.44
John L. Heid U. S. Food and Drug Inspection Station Cincinnati, O.	93.93	94.09	Sample coagulated	
J. W. Huntzinger H. J. Heinz Co. Pittsburgh, Pa.		94.65 94.59		26.11 26.07
O. S. Keener U. S. Food and Drug Inspection Station St. Louis, Mo.	93.82 93.81 93.98	93.26 93.41	26.22 26.36 26.50	26.84 26.76
Percy S. Eddy U. S. Food and Drug Inspection Station Minneapolis, Minn.	93.78 93.82	93.76 93.82	26.26 26.23	26.11 26.39
L. H. Bailey Bureau of Chemistry Washington, D. C.	93.97 93.97 93.98	94.18 94.22 94.24	25.89 25.91 25.85	26.07 25.98 26.07
R. L. Horst U. S. Food and Drug Inspection Station New Orleans, La.	94.20 94.22	94.09 94.07	26.17 26.12	26.08 26.01
R. S. Fleming Merrell-Soule Co. Syracuse, N. Y.	93.80 93.83 93.85	94.40 94.44	26.98 26.88 27.00	27.00 27.00 27.03
B. R. Jacobs Jacobs Laboratories, Inc. Washington, D. C.	93.17 93.40 93.44	94.00 93.94 93.96	26.26 25.87 25.94	26.61 26.10 26.46
Maximum	94.35	94.65	27.00	27.03
Minimum	93.17	93.26	25.85	25.98
Average	93.86	94.05	26.27	26.37

*Umpire Vacuum Method.*

Water added to make uniform layer;  
 Temperature of oven 100°C. throughout;  
 Pressure of oven 25 mm.;  
 Constant weight — 5½ hours.

	<i>per cent</i>
Sample No. 1.....	26.93
Sample No. 2.....	26.95
Average.....	26.94

## CONCLUSIONS.

The average results show that the umpire vacuum method and rapid

*determination in dried and liquid eggs.*

AVERAGE PRESSURE IN VACUUM OVEN	TEMPERATURE IN VACUUM OVEN	TYPE OF VACUUM OVEN	TEMPERATURE IN AIR OVEN	TYPE OF AIR OVEN
mm.	°C.		°C.	
30-35 (Manometer)	99	Cylindrical water- jacketed	113-115	A. H. T. varsity
25	99	Water- jacketed	112-116	Freas electric
No vacuum	oven			
56	98	E. & A. Cat. 1920, No. 4893	115-120 125	Single walled copper hot air oven
35 (Manometer)	99	Water- jacketed Bureau of Chemistry oven	112-115	Freas electric
12-13	98	Water- jacketed Bureau of Chemistry oven	112	De Khotinsky electric
13-17	98	Water- jacketed	114	Electric
25	100		112-113.4	
12-13	98	Water-jacketed Bureau of Chemistry oven.	112	De Khotinsky electric

routine method are in quite close agreement. However, there appears to be a considerable range in the results obtained by different collaborators. This is probably due to the difficulty of keeping the sample representative and could be overcome by asking each analyst to procure his own samples and apply the methods as given.

## RECOMMENDATIONS.

It is recommended that both the umpire vacuum and rapid routine methods for solids determinations on dried and liquid eggs be adopted as tentative.

No report on ash and water-soluble protein-nitrogen precipitable by 40 per cent alcohol and sampling of flaked eggs was given by the associate referee.

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No report on zinc in dried eggs was given by the associate referee.

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No report on acidity of fat and acid-soluble phosphoric acid was given by the associate referee.

### REPORT ON FOOD PRESERVATIVES.

By W. W. RANDALL (State Department of Health, Baltimore, Md.),  
*Referee.*

At the last meeting of this association the referee recommended that further study be given to the sublimation method for the separation, purification, and determination of benzoic acid and benzoates, salicylic acid, and saccharin.

An investigation of the possibilities of the method of sublimation, in which the apparatus devised by Hortvet was employed, was carried on during the past year whenever time could be found for the work. The substances studied were benzoic acid, salicylic acid, and ketchups containing benzoic acid or sodium benzoate. No work was done with saccharin.

The object of this preliminary work was to ascertain whether the material subjected to sublimation could be recovered with reasonable ease and completeness.

#### EXPERIMENTS.

##### *Experiment 1.*

*Benzoic acid:* C. P. benzoic acid (0.1300 gram) was weighed, dissolved in neutral alcohol, and titrated with standard soda solution and phenolphthalein.

Found (cold titration).....0.1262 gram = 97.10 per cent

The solution was then heated nearly to boiling, and the titration was continued.

Found (hot titration).....0.1281 gram = 98.53 per cent

The hot titration corresponded to a quantity of pure benzoic acid equal to 98.53 per cent of the weight of the C. P. benzoic acid used.

##### *Experiment 2.*

Of the same C. P. acid, 0.1618 gram was subjected to sublimation, the sublimate washed by means of ether into a dish, and the ether evaporated.

The residue in the sublimator-dish weighed, apparently, 0.0002 gram. The residue from the ether solution weighed 0.1631 gram—0.0013 gram more than the quantity started with. This residue was dissolved in neutral alcohol and titrated.

Found (cold titration)..... 0.1591 gram = 98.47 per cent

Found (hot titration)..... 0.1606 gram = 99.40 per cent

While washing the sublimate from the apparatus it was noticed that by mistake a sublimator had been used which had not been specially cleansed prior to the experiment. Traces of some deposit were still visible after the washing out with ether, and some minute particles were seen floating in the solution at the time of titrating. Hence, all the figures last given above are probably too high.

### *Experiment 3.*

*Salicylic acid:* Salicylic acid (1.3957 grams) was sublimed, washed from the apparatus into a dish, and the solvent evaporated. The residue in the sublimator-dish weighed 0.0013 gram; hence, the weight of material vaporized by heat was 1.3944 gram. On dissolving in neutral alcohol and titrating—

Found (cold titration)..... 1.3766 gram = 98.71 per cent

Found (hot titration)..... 1.3881 gram = 99.53 per cent

From the preceding results it may be fair to conclude that even when working with quantities of sublimable materials far greater than those for which the apparatus was designed, results of comparative accuracy may be secured. Much depends upon the skill with which the two portions of the sublimator have been ground to fit one another. So firmly had the two parts been forced together by the atmospheric pressure, in the case of the salicylic acid experiment, that they could not be separated except by a series of sharp taps with a hard-wood stick. Since the surface of the condensing bulb was festooned with long slim crystals attached at one end, such rough handling is naturally likely to detach portions of the sublimate with consequent possible loss.

On the other hand, when it is necessary to wash off only a few milligrams of deposit, distributed more or less evenly over a considerable surface, there is little excuse for error.

The next experiments were made upon the determination of sodium benzoate in ketchup. In this work the referee was assisted by W. H. Schulze. Three objects were in view:

(1) To compare the weight lost by the sublimator-dish, through sublimation of a part or all of its contents, with the weight of the residue left after evaporation of the solvent with which the sublimate had been washed from the apparatus;

(2) To compare these results with those secured by titration;



(3) To test these methods of determination in cases where only a very small quantity of benzoate was present.

*Sodium benzoate in ketchup—first series.*—A ketchup containing benzoate was extracted as usual, except that the volume of the solution was made up to 500 cc. after filtration and washing of the filters with salt solution. After evaporation of the chloroform, the crude residues in four parallel determinations weighed, respectively, per 100 cc. of solution extracted, 0.0330, 0.0291, 0.0343, and 0.0315 gram. Grease from separator stopcocks and, probably, some essential oil from spices, were present. These crude residues were subjected to sublimation. The greater part of the deposit formed between 100° and 115°C.; the temperature, however, was allowed finally to rise to 130°–140°C. before turning out the burner flame. The losses in weight of the sublimator-dishes and their contents were, respectively, 0.0208, 0.0207, 0.0211, and 0.0204 gram. The sublimed material was washed from the sublimator, and the solvent used was evaporated off; the residues weighed, respectively, 0.0206, 0.0197, 0.0216, and 0.0200 gram. Titration with standard soda solution and phenolphthalein was then carried out after solution of the benzoic acid in neutral alcohol. Determinations made by the referee are designated by R' and R'', those made by Schulze by S' and S''.

Benzoate found:	R'	R''	S'	S''
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
From weight of sublimed material	0.0810	0.0776	0.0829	0.0802
From loss in weight of sublimator-dish.	0.0818	0.0813	0.085	0.079
By titration. . . . .	0.0712	0.0708	0.0716	0.0712

The figures obtained by titration correspond to the first color change in the *cold* solution. It was found, however, that on standing the color disappeared and the addition of more soda was required to restore it; on heating the solution a further addition of soda was called for. While the final amount of soda required for a hot titration was not determined accurately in any case in this series, it was estimated that it would have corresponded to between 0.075 and 0.080 per cent in each case.

*Sodium benzoate in ketchup—second series.*—To each of two 100 gram portions of a ketchup said to contain no benzoate were added 15 grams of salt and 300 cc. of saturated salt solution. To one portion ("A") 10 milligrams, to the other ("B") 30 milligrams, of benzoic acid were then added, after which each was rendered alkaline. Through the use of such small quantities of the acid it was felt the accuracy of the work would be put to a severe test. After thorough mixing, each sample was passed through a muslin strainer and then through a double paper filter; the muslin and paper filters were then washed with saturated salt solution, in each case, until a final volume of 500 cc. of clear filtrate had been obtained. Extraction with chloroform and removal of the solvent by evaporation were conducted as usual. Four determinations of benzoic

acid were made with "A" and four with "B"—two each by the referee and two each by Schulze. In each case 100 cc. of acidified solution was extracted with chloroform. The following results were obtained:

	SOLUTION "A"		SOLUTION "B"	
	gram		gram	
Benzoic acid present . . . . .	0.0020		0.0060	
Found by titration (R) . . . . .	0.00143*	0.0010*	0.0056†	0.0062‡
Found by titration (S) . . . . .	0.0017†	0.0013†	0.0046*	0.0041*
Found by titration (S) . . . . .	...	...	0.0063†	0.0054†
From weight of sublimate (S) . . . . .	0.0026‡	0.0033‡	0.0055	0.0060

\* Results obtained in cold titration

† Results obtained in hot titration

‡ Many minute droplets of an oil were present—probably contained in the ether used to remove the acid from the sublimate. Results are therefore too high

### CONCLUSIONS.

So far as the work has gone, the following conclusions seem justified:

(1) The gravimetric determination of sodium benzoate in ketchup is as accurate as the determination by titration. By "gravimetric determination" is meant either (a) the weighing of the benzoic acid deposited by sublimation, according to Hortvet's method, or (b) the determination of the acid through a comparison of the weights of the sublimate-dish before and after the sublimation process.

(2) The titration of the benzoic acid with standard soda solution should be so conducted that the end point is determined when the solution of the acid is near the boiling point.

(3) In the determination of these preservatives by the sublimation method, it is obvious that care must be used to secure pure solvents and to remove traces of water or volatile acid from the material to be subjected to sublimation. On the other hand, the inclusion of some of the aqueous liquid with the chloroform during extraction is less likely to cause error when the *weight* of the sublimed material is determined than when the extracted acid is titrated by the official method.

### RECOMMENDATION<sup>1</sup>.

It is recommended that the study of the sublimation method for the separation, purification, and determination of benzoic acid, salicylic acid, and saccharin, when these or their derivatives are used as preservatives in food products, be continued during the coming year.

*R. E. Doolittle:* I would like to ask Dr. Randall if he experienced any difficulty in breaking or getting his apparatus apart.

*W. W. Randall:* The first piece of apparatus that was furnished by the manufacturers was too shallow to hold the dishes properly. After

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9, 84.

working with it for a while and finding that that was the case, I sent it back. Another difficulty seemed to be that of getting the apparatus apart again without jarring it in an undesirable way. The second and third apparatus that were sent to me by the manufacturers, however, did not seem to present the same difficulty, owing to the fact, perhaps, that we used some of the particular grease that they recommended. Formerly I had used vaseline according to the suggestion of Mr. Hortvet, and found it very difficult to get the apparatus apart. I found I had to use more of this grease than should have been necessary, but it didn't seem to get on the dishes which were to be weighed. It is a question, I presume, of very careful grinding. As a matter of fact, I have at times found it necessary to use a wooden stick and a hammer to dislodge the lower cup by tapping, and you can readily see that it might be easy to dislodge some of the sublimate.

## REPORT ON COLORING MATTERS IN FOODS.

By C. F. JABLONSKI<sup>1</sup> (U. S. Food and Drug Inspection Station, New York, N. Y.), *Referee*.

It is regretted that owing to the unusual press of regulatory work in the New York Station the referee was unable to carry out the recommendations of the committee<sup>2</sup>. However, there are submitted herewith results of some additional studies on yellow AB and yellow OB, particularly regarding their separation from each other in commercial oil mixtures.

If equal quantities of these two dyes are taken and made up in two exactly similar solutions in either 50 or 95 per cent neutral alcohol, it will be found that the freshly prepared solutions have the same colorimetric value reading in a Schreiner colorimeter. After long standing, however (two months or more), the solution of yellow AB becomes darker and increases in colorimetric value over 20 per cent.

To separate the dyes from an oil base (cottonseed oil), the following procedure was found adaptable:

Pipet 5 cc. of the cottonseed oil solution of the mixed dyes into a 100 cc. separatory funnel. Add 5 volumes of liquid petrolatum light U. S. P., and extract the mixture with 20 cc. of acetone. Allow to stand until the two layers are cleanly separated and then transfer the lower layer to a second funnel. It should be noted that if the acetone layers are not separated cleanly from the compound oil solution a considerable quantity of oil will interfere later. Re-extract the cottonseed oil petrolatum mixture with three or four 20 cc. portions of acetone, proceeding exactly as directed above. Ordinarily, four 20 cc. portions of acetone are sufficient to extract all coloring matter, but if the fourth portion of acetone should be colored, an additional fifth extraction with 20 cc. is necessary.

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<sup>1</sup> Presented by J. A. Cummings.

<sup>2</sup> *This Journal*, 1926, 8: 274.

Finally combine all acetone fractions into a small casserole and evaporate over a low steam bath. The residue from the casserole will contain the coloring matters with a very small quantity of oil.

Dissolve the coloring matter with two or three 10 cc. portions of normal hydrochloric acid and filter. Dilute the filtrate with water to approximately 100 cc. Place 40 cc. portions of low boiling petroleum ether in two separatory funnels and pass 25 cc. of the hydrochloric acid coloring solution successively through the petroleum ether in both funnels. Also pass the remainder of the hydrochloric acid color solution through these funnels in the same way, in 25 cc. portions.

Combine and wash the petroleum ether extracts with 10 cc. of water to remove traces of acid. Discard the water layer and transfer the petroleum ether extract of the color to a casserole and evaporate to dryness on the steam bath. Dissolve the residue which contains the mixed dyes in a pure state in 95 per cent alcohol, transfer to a 50 cc. volumetric flask, and make up to the mark with alcohol. Use 25 cc. of this alcohol solution for the quantitative determination of the total color by the method indicated below, and the remaining 25 cc. for the quantitative separation of the mixed colors, using the method submitted by the referee at the meeting of 1924<sup>1</sup>.

For determining the percentage of total color, the following method is suggested:

Pipet 10 cc. of a 0.04 per cent solution of yellow AB dissolved in 95 per cent alcohol into a 50 cc. volumetric flask and made to the mark with alcohol. (This is equivalent to 4 milligrams of dye.) Place 25 cc. of this solution into a Schreiner tube and made up to 50 cc. mark with 95 per cent alcohol. Into another Schreiner tube pour 25 cc. of the unknown solution and make up to 50 cc. mark with 95 per cent alcohol.

Compare the color intensity reading at every 5 millimeters from 10 to 40 millimeters. Combine the figures obtained and from them compute the amount of total dye present. If the intensity difference between samples is over 25 per cent, prepare another standard solution.

Referring to the method for the quantitative separation of colors reported last year, the accompanying table of results was obtained. It may also be used as a table of corrections to be applied in estimating the quantity of yellow OB.

QUANTITY TAKEN gram	QUANTITY RECOVERED gram
0.0004	0.00034
0.0008	0.00053
0.0012	0.00106
0.0016	0.0014
0.0020	0.0018
0.0024	0.0022
0.0028	0.0026
0.0032	0.0029
0.0036	0.0033
0.0040	0.0037
0.0048	0.0045
0.0060	0.0057
0.0080	0.0075
0.0120	0.0115
0.0160	0.0154

<sup>1</sup> *This Journal*, 1925, 8: 624.

The following figures were obtained in a similar manner for yellow AB:

QUANTITY TAKEN gram	QUANTITY RECOVERED gram
0.0004	0.00038
0.0008	0.00075
0.0012	0.00113
0.0016	0.0015
0.0020	0.0018
0.0024	0.0022
0.0028	0.0026
0.0032	0.0029
0.0036	0.0033
0.0040	0.0037
0.0048	0.0044
0.0060	0.0055
0.0080	0.0074
0.0120	0.0111
0.0160	0.0155

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That collaborative work be undertaken on the separation of light green SF yellowish from guinea green B and yellow AB from yellow OB.

(2) That additional work be done in the matter of separating yellow AB and yellow OB from other oil-soluble dyes.

(3) That the method for the quantitative separation of amaranth from tartrazene be undertaken owing to the unsatisfactory results usually obtained by the present method.

#### REPORT ON METALS IN FOODS.

By W. F. CLARKE (Bureau of Chemistry, Washington, D. C.), *Referee*.

During the past eighteen months or more articles of such importance in this field have been published that attention should be called to them. A most interesting contribution by Mallory<sup>2</sup> deals with the unsuspected chronic poisoning effects of copper as revealed by 10 autopsies in a total of 288 at the Boston City Hospital, from March, 1922 to March, 1923. Prior to the post-mortems, the injury had been suspected in only one case. Mallory evidently believes that copper present in alcoholic liquors was the cause of the poisoning in several cases and that copper absorbed due to exposure in industrial occupations involving such risks was the cause in some of the others. He notes especially that the liver was damaged in all of his cases. Mills<sup>3</sup> reports that brass cooking and serv-

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 84.

<sup>2</sup> *Am. J. Path.*, 1925, 1: 117.

<sup>3</sup> *J. Am. Med. Assoc.*, 1925, 84: 1326.

ing vessels are extensively used in Korea, and that frequently food is eaten after it has stood in them for several hours. In fact, from infancy to old age the use of foods containing copper persists. In the cases studied by Mills most of the findings reported by Mallory were not found; however, Mills found that cirrhosis is prevalent and common even among young people.

The harmfulness of lead and arsenic is too well known to need comment. Though well recognized also, attention may be called to the danger of injury due to the ingestion of foods containing zinc. Sale and Badger<sup>1</sup> reported in 1924 the finding of 229 mg. of zinc per liter in a certain lot of root beer. It had been found by others that this caused immediate vomiting by those that drank it.

That these and other so-called heavy metals occur naturally in foods is stated by various investigators. Furthermore, contamination may occur in commercial and domestic preparations. Hess, Supplee, and Bellis<sup>2</sup> report the finding of copper in human and bovine milk. According to Billeter and Marfurt's<sup>3</sup> statement that about 10 mmg. of arsenic per 100 grams of tissue is normal for the human body, arsenic must be present normally in foods. Palladin<sup>4</sup> notes that 31 elements have been found in ashes of plants grown under natural conditions, and McHargue<sup>5</sup> states that arsenic, antimony, cadmium, copper, manganese, zinc, nickel, cobalt, barium, and strontium are widely found. Prandi<sup>6</sup> and also von der Heide<sup>7</sup> report that copper is frequently present in wines. Cox<sup>8</sup> shows that arsenic in quite appreciable quantity is present in various kinds of food fish and in the urine of those eating such fish.

It should be mentioned also that it is well recognized that care should be taken in the use of insecticidal sprays to avoid danger of food poisoning.

The citations given show that there is need for thorough study of sensitive and accurate methods for the determination of these metals, but since his appointment the time available for such work has only permitted the referee to shape up a previously unpublished colorimetric method for lead. It is similar to some other methods in which the organic food material is destroyed by wet combustion, the sulfate is precipitated in 57 per cent alcohol (by volume), and other interfering metallic sulfates are washed from the filtered precipitate with the same solvent. However, the referee found difficulty in the use of ammonium acetate in the colorimetric determination and had to seek another solvent for the lead sulfate. One that has given gratifying results is ammonium thiocyanate,

<sup>1</sup> *Ind. Eng. Chem.*, 1924, 16: 184.

<sup>2</sup> *J. Biol. Chem.*, 1923, 57: 725.

<sup>3</sup> *Helvetica Chim. Acta*, 1923, 6: 780.

<sup>4</sup> *Plant Physiology*, 2nd ed., 1923.

<sup>5</sup> *J. Agr. Research*, 1925, 30: 193.

<sup>6</sup> *Stat. sper. agrar. ital.*, 1921, 54: 469.

<sup>7</sup> *Z. anal. Chem.*, 1925, 66: 24.

<sup>8</sup> *Analyst*, 1925, 50: 3.

but so far as he has been able to review the literature it has not been reported as useful for the present purpose. Of the solution in the thiocyanate, all or a suitable aliquot is compared colorimetrically with standards containing lead acetate to which has been added the same quantity of thiocyanate as is contained in the unknown, the total volume being the same.

The procedure used was as follows:

In the presence of 10 mg. each of copper, ferric iron, and zinc, and with 5 cc. of concentrated sulfuric acid added, known quantities of lead acetate were heated to fuming. After cooling, 100 cc. of 57 per cent alcohol (by volume) was added, and the mixture was allowed to stand overnight. After stirring so as to dissolve any copper sulfate, the precipitate was filtered off on doubled filter paper, C. S. & S. 589 blue ribbon, and washed practically free from the other metals (a trace of iron is not harmful). The lead sulfate was dissolved in 20 cc. of ammonium thiocyanate (20 per cent), but 40 cc. was used for the 5 mg. lead sample. To each solution in Nessler tubes a 10 cc. portion of hydrogen sulfide water was added, and colorimetric comparisons were made with a series of known lead acetate solutions to which had been added the same quantities, respectively, of the ammonium thiocyanate solution and hydrogen sulfide water.

The samples marked (a) were treated as described, but they contained 5 grams of aluminium sulfate each. In the sample marked (b), the ammonium thiocyanate solution of the lead sulfate was diluted to 100 cc. and 1 cc. of this solution, added to 20 cc. of the ammonium thiocyanate solution, was used for the estimation.

The preliminary results are shown in the table.

LEAD PRESENT mg.	LEAD FOUND mg.
0.03	0.02+
0.03	0.02—
0.03 (a)	0.02—
0.05	0.05—
0.05 (a)	0.04+
0.00	0.0025—
0.00 (a)	0.0025
0.00 (a)	0.0025—
0.03	0.03
0.03	0.02
0.05	0.05
0.05	0.05
0.00	0.002—
0.00	0.002—
5.18 (b)	5.18+

Summarized, it appears that this method is sufficiently accurate for at least as much as 5 mg. and as little as 30 mmg. of lead. Iron, zinc, copper, and aluminium may be present to the extent of at least 10 mg. of each. The blank also is shown to be negligible.

#### RECOMMENDATIONS.

It is recommended—

- (1) That more sensitive methods be sought for tin, copper, zinc, and

aluminium, and that any such method found be studied collaboratively.

(2) That the proposed method for lead be studied collaboratively.

*J. G. Lipman:* Dr. Browne was kind enough to give me an opportunity to say a few words this morning concerning the coming International Congress of Soil Science which is to be held in Washington in June, 1927. As you are all interested in soil problems, I should like to have you get acquainted with the work of the proposed congress. A group of men met at Budapest in 1909 and organized what was called the First International Conference of Soil Science. The scope of this congress was enlarged when another international meeting was held at Stockholm in 1911. In 1922 the Third International Conference of Soil Science was held in Prague. At that time problems relating to the application of soil science to land utilization were included. The last session was held in Rome in 1924. Here it was decided to organize an international society and to hold the next congress in the United States.

One of the features of the congress will be a field excursion which will proceed along a definitely determined itinerary. Dr. C. F. Marbut of the Bureau of Soils will have suitable pits dug so that the delegates may be able to see the soil profiles of the United States. There will be an opportunity given to become acquainted with the agricultural industries of the United States—the fertilizing industry, the packing industry, canning, cheese and butter making, etc. I thought that it might be well to make this preliminary announcement to you and suggest that if any of you are interested we shall be very glad to answer any questions or give any further information.

## REPORT ON FRUITS AND FRUIT PRODUCTS.

By P. L. GOWEN (Bureau of Chemistry, Washington, D. C.)<sup>1</sup>, *Referee*.

The association approved six recommendations pertaining to work to be conducted this year on the subject of Fruits and Fruit Products. The recommendations are:

(1) That methods for the determination of fruit ash in the case of fruit products containing added non-volatile ingredients like phosphoric acid, alum, or calcium salts be further studied.

(2) That a study of the determination of malic acid in the presence of citric and tartaric acids be undertaken.

(3) That the official method for tartaric acid be made the subject of further study for the purpose of improving its accuracy.

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<sup>1</sup> Present address: National Canners Association, Washington, D. C.



(4) That the method for determining added water in grape juice be presented for collaborative work during the coming year.

(5) That further study of the official method for the determination of commercial glucose<sup>1</sup> in jams, jellies, and preserves be made.

(6) That the refractive index method for soluble solids in fruit products be further studied with a view to substituting it for the drying method.

H. J. Wichmann, U. S. Food and Drug Inspection Station, San Francisco, Calif., and E. K. Nelson, Bureau of Chemistry, Washington, D. C., were appointed associate referees. In addition, B. G. Hartmann, the previous referee, consented to act as associate referee. Wichmann undertook to give general attention to the compilation of methods best adapted to the analysis of fruit ash. He reported that he supervised ash analyses on a variety of fruits and made a study of the most favorable methods. Owing to press of other work, however, he was not able to compile these methods satisfactorily and will hold them over until next year. Nelson continued his work on the determination of organic acids, devoting most of his time to a method for inactive malic acid and giving incidental attention to the determination of tartaric acid. The direction of the collaborative work of Recommendation (4), added water in grape juice, was undertaken by Hartmann. Lack of time prevented a further study of the present official tartaric acid method<sup>2</sup> that he had planned.

The referee gave attention to Recommendations (5) and (6).

#### DETERMINATION OF COMMERCIAL GLUCOSE IN JAMS AND JELLIES.

The glucose used was the ordinary confectioners' brand with a solids content of 85.1 per cent as determined by the refractometer. The jellies were prepared from currant juice, sucrose, liquid apple pectin solution, and known weights of the commercial glucose. The jellies were boiled to 105°–106°C., and the finished product was weighed before being poured into the glasses. The jams were prepared from canned peaches, sucrose, and known quantities of the commercial glucose. The fruit and sugar solids were in the ratio of 45:55, and the batches were evaporated to a definite weight to secure a resultant product of the usual commercial consistency.

The method for commercial glucose in the Fruits and Fruit Products chapter of the official methods refers to the method given in the chapter on Sugars and Sugar Products. Since this method further refers to the preparation of the solution and inversion procedure most suitable to sugars and sugar products, it was considered advisable to detail the method for the preparation of the solution best adapted to fruits and fruit products and repeat the glucose method. Consequently the following directions were sent to the collaborators:

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 216.

<sup>2</sup> *Ibid.*, 213.

## COMMERCIAL GLUCOSE (APPROXIMATE).

## PREPARATION OF SAMPLE.

*Jellies*.—Mix thoroughly to insure uniformity in sampling.

*Jams*.—Pulp by grinding in a mortar or by passing through a food chopper and mix thoroughly.

## DETERMINATION.

Mix the normal weight (26 grams) of the sample in water in a 100 cc. flask, using heat if necessary to dissolve the sugars. Add an excess of normal lead acetate solution and 1–2 cc. of alumina cream if necessary, shake, dilute to the mark with water, mix well, and filter. Remove the lead from the filtrate with anhydrous potassium oxalate or anhydrous sodium carbonate. Pipet a 50 cc. portion of the lead-free filtrate into a 100 cc. flask. Add 5 cc. of concentrated hydrochloric acid and complete the inversion by setting aside for 24 hours at a temperature not below 20°C., or, if the temperature is above 25°C., set aside for 10 hours. The quicker method by acid hydrolysis<sup>1</sup> may be

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 186, 23(b).

followed if desired.

After inversion make neutral to phenolphthalein with sodium hydroxide solution; slightly acidify with dilute hydrochloric acid; and treat with 5–10 cc. of alumina cream, if necessary, before making up to the mark. Filter, if necessary, and polarize at 87°C. in a 200 mm. jacketed metal tube, preferably silver. Multiply the reading by 200 and divide by the factor 196 to obtain the percentage of commercial glucose solids polarizing +211°V.

*Report*

- (1) Invert reading at 87°C. normal solution.
- (2) Percentage of commercial glucose solids polarizing +211°V.

The results obtained are given in Table 1.

Taking into consideration the variable composition of commercial glucose, which makes the polarization method for its determination an approximate one only, the results of the collaborators are considered good; they indicate that the official method for commercial glucose is applicable in the examination of jams and jellies. It is of interest to note Palmore's results, showing that the basic lead acetate may be used as the clarifying agent.

## DETERMINATION OF TOTAL SOLIDS IN PRODUCTS CONTAINING SUCROSE AND ORGANIC ACIDS.

In preliminary work reported in the 1924 meeting<sup>1</sup>, Associate Referee Wichmann showed that there is a material error in the use of the drying method for the determination of solids in concentrated solutions of sucrose containing small percentages of organic acids. He further showed that this was due to the inversion of the sucrose and that very accurate results could be secured with the refractometer.

The referee continued this work during the past year, also working with synthetic solutions of sucrose and organic acids. The results of last

<sup>1</sup> *This Journal*, 1925, 8: 629.

TABLE 1.  
*Collaborative results on determination of glucose.*

COLLABORATORS	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4		SAMPLE 5	
	Polariza- tion 87°C. Normal Solution	Glucose Solids	Polariza- tion 87°C. Normal Solution	Glucose Solids	Polariza- tion 87°C. Normal Solution	Glucose Solids	Polariza- tion 87°C. Normal Solution	Glucose Solids	Polariza- tion 87°C. Normal Solution	Glucose Solids
JELLIES		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>		<i>per cent</i>
		0.0		4.7		9.5		25.3		54.4
	-0.82*	....	+8.6*	4.39*	+18.2*	9.28*	+50.0*	25.51*	+107.9*	55.06*
	-0.80*	....	+8.0*	4.08*	+17.9*	9.13*	+49.4*	25.26*	+106.9*	54.57*
	+0.4†	....	+6.8†	3.5†	+18.4†	9.4†	+48.2†	24.6†	+104.4†	53.3†
JAMS	+0.4†	....	+7.2†	3.7†	+16.4†	8.4†	+48.4†	24.7†	+107.2†	54.7†
	-0.8	....	+8.0	4.08	+17.0	8.67	+49.6	25.31	+103.6	52.86
	-0.8	....	+8.0	4.08	+17.2	8.78	+49.8	25.41	+103.6	52.86
		0.0		9.3		3.9		41.8		21.7
	-0.42	....	+18.4	9.39	+7.20	3.67	+84.10	42.91	+43.64	22.27
JAMES	-0.60	....	+18.6	9.49	+7.00	3.57	+84.56	43.14	+42.82	21.85
	0.0	....	+17.6	8.98	+9.0	4.59	+83.0	42.35	+45.2	23.06
	+0.2	0.1	+17.8	9.08	+8.4	4.29	+82.8	42.25	+44.6	22.76
	+3.6	1.85	+20.6	10.56	+8.8	4.51	+75.4	38.67	+36.8	18.87
	+2.8	1.44	+18.8	9.64	+9.0	4.62	+72.6	37.23	+37.8	19.39

\* Basic lead acetate used as clarifier.  
† 0.5N original jelly solution used.

year by the drying method were determined by weighing into an aluminum dish, thinning with water, evaporating the excess on the steam bath, and finally drying to constant weight in vacuo at 70°C. Since the official method calls for the use of either pumice stone or sand, the latter and also different quantities of asbestos were used in the drying determinations. Samples of 1-2 grams were weighed directly into aluminum dishes of 2½ inches diameter provided with closely fitting covers. Short glass rods were included in those containing sand and asbestos. All samples were well distributed in the dishes, introduced directly into the vacuum oven at 70°C., and dried to constant weight. Reducing sugars were determined on the dried residue after taking up with water by the Munson and Walker method.

The results are given in Table 2.

TABLE 2.

*Results of determinations of solids and invert sugar in dried residue.*

METHOD USED	SOLUTION NO. 1		SOLUTION NO. 2	
	500.00 grams of sucrose 1.00 gram of tartaric acid 49.83 grams of water		Same as Solution No. 1 Prepared for check on previous results	
	Solids	Invert Sugar in Dried Residue	Solids	Invert Sugar in Dried Residue
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Theoretical . . . . .	50.57	....	50.57	.. .
By Abbé refractometer (Geerlig's table) . . . . .	50.58	...	50.61	.
Drying at 70°C. Vacuo—				
No asbestos used—no water added . . . . .	54.16	36.2	54.0	35.1
No asbestos used—water added . . . . .	53.16	29.3	53.0	32.0
½ gram asbestos used—no water added . . . . .	51.51	11.1	51.34	9.4
½ gram asbestos used—water added . . . . .	51.27	7.8	51.69	11.2
5 grams asbestos used—no water added . . . . .	51.04	7.6	51.33	.. .
5 grams asbestos used—water added . . . . .	51.00	5.5	51.4	10.3
10 grams asbestos used—no water added . . . . .	....	....	51.42	13.6
10 grams asbestos used—water added . . . . .	.	.	51.28	..

The figures in Table 2 support Wichmann's conclusions that results by the drying method are too high. However, when asbestos or sand is used, much less inversion takes place, and the results are nearer the theoretical solids content. It will be noted that the inversion of the sucrose does not account for the total error in those samples where neither asbestos nor sand was used; these samples apparently still contained moisture and were losing weight after 21 hours in the oven. The asbestos was most satisfactory, as practically without exception constant weight was secured in 4-6 hours' drying. Since in manufactured fruit products containing sucrose and organic acids, partial inversion has already taken

place, a comparison of the results by the refractometric and drying methods was made on a series of synthetic solutions that had been partially inverted. These solutions contained approximately 1 per cent tartaric acid and varying percentages of the sugars. The results are given in Table 3.

TABLE 3.  
*Comparison of results by refractometric and drying methods.*

SOLIDS	1	2	3	4	5	6	7
Refractometer.....	18.25	29.82	38.67	45.37	50.97	41.52	38.92
Vacuo 70°C. (asbestos) ..	18.01	29.90	38.61	45.57	50.94	41.60	38.97

The results on a single series of solutions indicate that where the product is partially inverted solids by the refractometer and drying at 70°C. in vacuo agree very well. It is recommended that collaborative work on similar solutions be carried on next year. Investigators in sugar analysis have shown that the refractometric method for soluble solids gives accurate results with pure solutions of the various sugars. The indices of refraction of the fruit acids are very close to that of the sugars so that it would seem that further investigation is not needed to show the accuracy of the refractometer method for such synthetic solutions, regardless of the amounts of inversion. The work recommended would indicate any error in the present official drying method. If no error is conclusively shown, it is probable that the drying method will give the more accurate results on fruits and fruit products since these contain material quantities of non-sugar, non-acid solids which might render the refractometric method inaccurate.

#### METHODS.

Since the last meeting the second revision of *Methods of Analysis* has appeared. As with the previous issue, many of the methods in the various chapters are handled by reference to other chapters. While this is usually satisfactory, it is believed that in many cases the technique best adapted to the specific products under discussion, such as preliminary treatment of the samples, preparation of the solutions, etc., could profitably be given in all cases and the method itself repeated in some cases. An example is the glucose determination studied this year. Referring now to the Fruits and Fruit Products chapter in the 1925 edition, the following comments are made on the paragraphs designated:

**2 Alcohol (Official).** This reference is to the method used in alcoholic confectionery sirups. Since the presence of alcohol in fruit products is generally associated with a fermentation in which volatile acids may be formed, it is believed that a reference ahead to p. 327, 17, which is the

method for alcohol in vinegars, would be more fitting. This method calls for making the solution alkaline before distillation.

**12 Chlorides (Official).** This reference is to chlorides in the ash and should be so indicated, as the method gives erroneous results for chlorides in a saccharine product as pointed out by Browne and Gamble<sup>1</sup>.

**27 Sucrose—by Polarization (Official).** The references here are to methods calling for inversion by (1) invertase, (2) hydrochloric acid. The second method is a tentative one in the same chapter, and it is stated that erroneous results are secured by it in products containing much levulose, including fruit products. Apparently this method should be omitted or the magnitude of the error involved stated so that the analyst may judge whether it is significant in his work. Regardless of the accuracy of the first method, analysts in ordinary food work will prefer the hydrochloric acid method because of its great simplicity. Jackson and Silsbee<sup>2</sup> state that reliable analyses for sucrose may be obtained in solutions containing invert sugar if the direct polarization is made in the presence of a weight of sodium chloride equal to that of the hydrochloric acid used for the invert polarization. This slight modification of the second method referred to will apparently eliminate the error involved and should be investigated by the association.

**27 Sucrose (by polarization),**

**28 Sucrose (by reduction), and**

**29 Reducing sugars.**

Directions for the preparation of solutions so as to minimize any inversion of the sucrose by unnecessary heating should be included in the three preceding methods.

#### RECOMMENDATIONS<sup>3</sup>.

It is recommended—

(1) That the method for the determination of i-malic acid be further studied with a view to subjecting it to collaborative work.

(2) That the method for the determination of added water in white grape juice as modified in the report of the associate referee be submitted to collaborative study.

(3) That a further comparison be made of the refractometric and official vacuum methods for solids in solutions containing sucrose and organic acids.

(4) That methods best adapted for the complete analysis of the ash of fruit products be compiled with a view to including them in the official methods.

<sup>1</sup> Facts About Sugar, 1923, 18: 552.

<sup>2</sup> Bur. Standards Tech. Paper, 259, p. 281.

<sup>3</sup> For report of Sub-committee C and action by the association, see *This Journal*, 1926, 9: 85.

(5) That attention be given to all methods in the chapter on Fruits and Fruit Products now handled by reference and directions submitted to the association at its next meeting where these are necessary to make the methods definite and complete.

## REPORT ON ADDED WATER IN GRAPE JUICE.

By B. G. HARTMANN (Bureau of Chemistry, Washington, D. C.),  
*Associate Referee.*

During the preparation of the second edition of *Methods of Analysis* the writer recommended to the chairman of the Committee on Revision of Methods of Analysis that the procedure for determining added water in Catawba grape juice, read before the 1924 meeting of the association, be included as a tentative method. The chairman concurred in the recommendation, provided the writer assume full responsibility for the method, act as associate referee on this subject, and submit the method to collaborative study during the coming year.

Based upon his past experience, the writer did not hesitate to assume responsibility for the procedure and agreed to the conditions specified. Accordingly, the procedure was published as a tentative method in the chapter on Fruits and Fruit Products.

Five samples of Catawba grape juice, carefully prepared and containing known quantities of 20 per cent sugar solution, together with copies of the procedure as given in the last edition of *Methods of Analysis*<sup>1</sup>, were submitted to six collaborators. Previously the writer had examined them for water content and obtained satisfactory results.

The first results submitted by three collaborators were not satisfactory for all the samples represented. From a careful consideration of these reports it was found that almost invariably the poor results were obtained when the temperature maintained during the procedure deviated materially from 25°C. It was concluded that the unfavorable results were due, not to any fundamental unsoundness of the procedure, but rather to a lack in the text of specific instructions regarding proper temperature control. As this conclusion made further reports unnecessary, the other collaborators were informed that no reports were expected from them.

Experiments were conducted by the associate referee for the purpose of obtaining closer temperature control. It was found that by wrapping the Mason jar containing the sample in heavy paper before placing it in the shaking machine the temperature could be held within a degree either side of the initial temperature. It was also found more desirable

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 218.

to adjust the temperature of the sample to exactly 25°C. before placing in the shaking machine than to operate at room temperature as directed in the present procedure.

These changes, it is believed, will not only improve the accuracy of the method, but they will make it applicable to the most variable temperature conditions.

No time was afforded for submitting the altered method to collaborative study. It is proposed to substitute the following text for the one given in *Methods of Analysis*, and it is recommended that the method in its modified form be subjected to collaborative study during the coming year.

#### THE MODIFIED METHOD.

Transfer about 50 cc. of the filtered juice to a 2 ounce tincture bottle containing ten pieces of glass roding, 15 mm. long and 5 mm. in diameter, and approximately 1 gram of finely powdered cream of tartar. Cork the bottle tightly and place it neck downward in a pint Mason jar. Fill the jar with water at 25°C. and hold at this temperature for one-half hour, shaking occasionally. Then seal the jar; wrap it in three sheets of heavy wrapping paper, making three separate wrappings; place in a mechanical shaker; and shake for 1 hour. After the shaking, ascertain the temperature, " $t^\circ$ ", of the water in the Mason jar. Filter the juice immediately and titrate 10 cc. with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator. Repeat the titration with 10 cc. of the original juice. Make the two titrations side by side in order to obtain the same shade of pink with the greatest possible accuracy. For calculating the percentage of added sugar solution (water), use the following formula:

$$W = \frac{0.0188(b-a) - 0.095 - 0.025 \left( \frac{t^\circ - 25}{2} \right)}{0.006}$$

$W$  = Percentage by volume of added water (20 per cent sugar solution).

$b$  = Acidity of treated juice, cc. 0.1 *N* sodium hydroxide per 100 cc.

$a$  = Acidity of original juice, cc. 0.1 *N* sodium hydroxide per 100 cc.

$t^\circ$  = Temperature after shaking.

Pure factory juices examined by this method show a small quantity of added water varying from 1 to 3 per cent.

No report on pectin in jams, jellies, and preserves was given by the associate referee.

#### REPORT ON FRUIT ACIDS.

By E. K. NELSON (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

The work done by the associate referee on fruit acids for the past year comprises a study of the separation and determination of tartaric acid as potassium acid tartrate and the separation and determination of



malic acid, both active (polarimetrically and gravimetrically), and inactive (gravimetrically), in the same solution.

In view of the fact that inactive synthetic malic acid has recently been put on the market and exploited as suitable for use in foods, it seems desirable to devise methods for its detection and estimation as the polarimetric method is, of course, inapplicable for this purpose. The gravimetric method to be described will work equally well with the natural (active) malic acid.

#### SEPARATION AND ESTIMATION OF TARTARIC ACID AND ESTIMATION OF I-MALIC ACID IN FILTRATE POLARIMETRICALLY.

The tartaric acid was precipitated in 25 cc. of aqueous solution containing potassium acetate and 0.5 cc. of acetic acid by adding 25 cc. of alcohol, stirring, and allowing to stand over-night in the ice box.

The filtrate from the potassium acid tartrate was concentrated, neutralized, made slightly acid with acetic acid, and made to 50 cc. volume. The optical rotation of this solution was taken in a 200 mm. tube, and 25 cc. was treated in the manner described in the tentative method<sup>1</sup> with uranyl acetate and polarized, the increase in rotation being calculated to i-malic acid.

The results of experiments are given in Table 1.

TABLE 1.  
*Determination of tartaric and malic acids in the same solution.*

TAKEN				RECOVERED	
Citric Acid	Tartaric Acid	Malic Acid	Potassium Acetate	Tartaric Acid	Malic Acid
<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>	<i>gram</i>
....	0.25	....	0.4	0.2473	....
0.25	0.1	0.25	1.0	0.093	0.225
0.25	0.1	0.25	1.0	0.105	0.223
0.25	0.05	0.25	1.0	0.052	0.223
0.25	0.25	0.10	1.0	0.261	0.082
0.25	0.25	0.05	1.0	0.259	0.04
0.25	0.25	0.02	1.0	0.256	0.016
0.25	0.02	0.25	1.0	0.015	0.224

The method was then applied to the barium salts separated according to the tentative method<sup>2</sup>, the precipitate being treated in the following manner: Digest the barium salts with a solution of 2 grams of anhydrous sodium sulfate and filter. To the filtrate add 12.5 cc. of normal sulfuric acid and 1 gram of potassium acetate, and evaporate to dryness. Add 25 cc. of water, 0.5 cc. of acetic acid, and 25 cc. of alcohol; stir, and keep

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 213.

<sup>2</sup> *Ibid.*, 214.

in the ice box overnight. Filter and wash the acid tartrate with 95 per cent alcohol and titrate with 0.1 N sodium hydroxide. Estimate i-malic acid in the filtrate as described previously.

TABLE 2.

*Determination of malic and tartaric acids in the barium precipitate.*

TAKEN			FOUND	
Citric	Tartaric	i-Malic	Tartaric	i-Malic
gram	gram	gram	gram	gram
0.25	0.10	0.25	0.093	0.225
0.25	0.20	0.25	0.199	0.223
0.25	0.20	0.10	0.20	0.086

#### SEPARATION OF TOTAL ACIDS BY LEAD ACETATE.

The next series of experiments comprised a study of the separation of the total acids by means of lead acetate.

The acids were precipitated with 20 cc. of 35 per cent lead acetate solution and 1 liter of alcohol and allowed to stand several hours; the lead salts were filtered off and washed with alcohol. The paper and precipitate were placed in a flask with water and some glass beads to break up the precipitate, and hydrogen sulfide was passed to saturation. The excess of hydrogen sulfide was driven out with carbon dioxide, the lead sulfide was filtered off and washed, and the filtrate was concentrated to 25 cc. One gram of potassium acetate, 0.5 cc. of acetic acid, and 25 cc. of alcohol were added, and the mixture was stirred and kept overnight in the ice box. The further manipulation was the same as in the previous experiments. The results are given in Table 3.

TABLE 3.

*Determination of tartaric and malic acids in the lead acetate precipitate.*

TAKEN			FOUND	
Tartaric	i-Malic	Citric	Tartaric	i-Malic
gram	gram	gram	gram	gram
0.20	0.25	0.25	0.196	0.22

As the tentative method for the determination of malic acid, depending, as it does, on the optical behavior of natural (levo) malic acid, is useless for the determination of inactive synthetic malic acid which has recently come on the market, it is desirable to have a method for the determination of this acid.

Separation of soluble malates from insoluble citrates and tartrates, the conversion of malic acid into fumaric acid and its extraction with ether, and conversion of malic into fumaric acid and the oxidation of this acid to racemic acid by means of a catalyst, all suggested themselves.

#### SEPARATION OF SOLUBLE LEAD MALATE FROM LEAD CITRATE AND LEAD TARTRATE.

Following a suggestion of Hartsen<sup>1</sup>, some experiments were undertaken to separate malic acid from other acids by taking advantage of the greater solubility of lead malate in dilute acetic acid at 60°–70°C. The acids, in 25 cc. of water, were treated with 7 cc. of 35 per cent lead acetate solution and 1 cc. of glacial acetic acid, heated an hour on the steam bath at 60°–70°C., stirred frequently, filtered on a Gooch crucible, and washed with 5–10 cc. of water heated to 70°C. The filtrate was decomposed with hydrogen sulfide and filtered from lead sulfide, and the filtrate was evaporated in a tared vessel, dried at 50°C. in vacuo, and weighed. The results are shown in Table 4.

TABLE 4.  
*Results of trial of the Hartsen method.*

TAKEN		FOUND
Malic (synthetic)	Citric	Malic
<i>gram</i>	<i>gram</i>	<i>gram</i>
0.05	0.25	0.05
0.10	0.25	0.09
0.25	0.25	0.20
In the following experiments the residue of acid was titrated instead of being weighed.		
0.25	none	0.25
none	0.25	0.014
0.15	0.05	0.17
0.02	0.25	0.03
0.25	0.02	0.27

As a preliminary means of separation it would appear that a method that depended upon the greater solubility of lead malate in dilute acetic acid might prove useful, although operating on complex mixtures, such as fruit juices, the results would be totally misleading without other means of purification and characterization of the malic acid. Therefore, experiments were carried out on the conversion of malic acid into fumaric acid and the oxidation of the fumaric acid to racemic acid. For the conversion of malic acid into fumaric acid, a modification of the method of Kunz<sup>2</sup> was used.

<sup>1</sup> *Arch. Pharm.*, 1875, 6: 110.

<sup>2</sup> *Z. Nahr. Genussm.*, 1903, 6: 728.

In Kunz's method the barium precipitate (or the mixture of acids) is evaporated with 10 cc. of 10 per cent sodium carbonate and 10 cc. of 10 per cent sodium hydroxide, and the residue is heated at 130°C. for 3 hours. The mass, dissolved in water, is acidified and thoroughly extracted with ether in a continuous extraction outfit (such as Palkin's<sup>1</sup>). The residue from the ether is dried and weighed as fumaric acid. By this method 0.25 gram of i-malic acid afforded 0.1996 grams of fumaric acid, equivalent to 0.227 gram of malic acid, a yield of 90.8 per cent. Treated in the same way, 0.25 gram of citric acid gave a residue of 0.0206 gram. Therefore, the presence of citric acid would interfere with the determination of malic acid by this method. As the major portion of the citric acid may be separated by the Hartsen method, its influence may be eliminated. However, there will be difficulty in properly characterizing the fumaric acid as thus far no satisfactory insoluble salts of it have been found. Later it was found that fumaric acid was formed in better yield by adding 2 molecules in excess of sodium hydroxide and heating 3 hours at 130°C. Sodium carbonate is not added when operating on the free acids. Its use is required when operating on the barium precipitate.

By the Kling method<sup>2</sup> for tartaric acid, a difficultly soluble calcium racemate is obtained, and if the fumaric acid can be quantitatively oxidized to racemic acid, the calcium salt of this can be precipitated and weighed. This has been accomplished by Milas and Terry<sup>3</sup>, who used osmium tetra-oxide as a catalyst. These workers used their own method for the preparation of racemic acid, but it would appear also to lend itself to analytical work, as they obtained yields as high as 99.5 per cent.

A preliminary experiment was carried out on pure i-malic acid as follows: 0.25 gram of i-malic acid was converted into fumaric acid by evaporating with 2 molecules in excess of sodium hydroxide and heating 3 hours at 130°C., and the residue of fumaric acid (obtained by ether extraction in a Palkin extractor), in 25 cc. of water, was mixed with 3 grams of potassium chlorate and 2 cc. of a 1 per cent solution of osmium tetra-oxide in a flask provided with an air condenser, and held at a temperature of about 50°C. overnight.

The solution was cooled, and the osmium tetra-oxide was recovered by extraction with benzene<sup>4</sup>. To the aqueous solution were added 50 cc. of a solution of 16 grams of calcium carbonate and 120 grams of acetic acid to the liter; it was then stirred and allowed to stand 2-3 hours, and the calcium racemate was filtered on a tared Gooch, dried at 100°C., and weighed. The calcium racemate weighed 0.3329 gram, equivalent to 0.237 gram of malic acid, a yield of 95 per cent. In the same manner,

<sup>1</sup> *Ind. Eng. Chem.*, 1925, 17: 612.

<sup>2</sup> *Bull. soc. chim.*, 1910, 7: 567; 1912, 11: 886.

<sup>3</sup> *J. Am. Chem. Soc.*, 1925, 47: 1412.

<sup>4</sup> *Ibid.*, 1414.

0.05 gram of malic acid yielded 0.0654 gram of calcium racemate, equivalent to 0.047 gram of malic acid.

#### SEPARATION OF FRUIT ACIDS FROM FRUIT PRODUCTS.

In order to get the best results from any method for the estimation of fruit acids, their separation from interfering substances, such as sugars, phosphoric acid, inorganic salts, etc., is a desideratum. Preliminary experiments with malic acid indicated that such a separation can be expeditiously accomplished through the use of the Palkin extractor.

Twenty-five cc. of a 1 per cent solution of i-malic acid, extracted for 14 hours with ether in the Palkin extractor, gave a recovery of 0.247 gram of malic acid, an efficiency of 98.8 per cent. Accordingly, a series of experiments was carried out in which the methods herein described were applied to the residues obtained by ether extraction of mixtures of black raspberry juice, sweetened, with known quantities of i-malic, tartaric, and citric acids. Black raspberry juice was chosen because investigation has shown its freedom from malic and tartaric acids<sup>1</sup>.

In the following experiments the potassium acid tartrate was titrated, and the malic acid in the filtrate from this titration was converted into racemic acid and weighed as calcium racemate.

TABLE 5.

*Determination of malic acid as calcium racemate after separation of tartaric acid as potassium acid tartrate.*

TAKEN						RECOVERED	
Juice	Tartaric	i-Malic	Citric	Time Ex- traction	H <sub>2</sub> SO <sub>4</sub>	Tartaric	Malic
cc.	gram	gram	gram	hours		gram	gram
25	0.25	0.10	0.25	10	1 dp.	0.2475	0.013
25	0.25	0.25	0.25	10	1 dp.	0.2535	0.127
25	0.10	0.10	0.25	15	1 cc.	0.08	0.03
50	0.10	0.10	0.25	30		0.085	not deter- mined
50	0.25	0.25	0.25	30		0.248	not deter- mined
25	0.10	0.10	0.25	24	1 cc.	not deter- mined	0.06
25	0.25	0.25	0.25	24	1 cc.	not deter- mined	0.23

In the following experiments the tartaric acid was separated and estimated as potassium acid tartrate, and the i-malic acid in the filtrate was converted into fumaric acid and then oxidized to racemic acid, which was precipitated and weighed as calcium racemate. The solution from which the acids were extracted was more strongly acidified than in previous experiments; therefore, at the risk of repetition, the procedure is described in full as follows:

<sup>1</sup> *J. Am. Chem. Soc.*, 1925, 47: 1177.

Extract 25 cc. of sweetened black raspberry juice, 2 cc. of sulfuric acid, and the weighed added acids for 24 hours in the Palkin extractor. Evaporate the ether after the addition of 10 cc. of 10 per cent potassium acetate solution and bring to dryness. Add 25 cc. of water, 0.5 cc. of acetic acid, and 25 cc. of alcohol; stir the mixture, and keep in the ice box overnight. Filter the potassium acid tartrate on a Gooch, wash with a little 95 per cent alcohol, dissolve in hot water, titrate with 0.1 *N* sodium hydroxide, and calculate to tartaric acid. Evaporate the filtrate from the acid tartrate until most of the acetic acid is dispelled, again evaporate with 10 cc. of 10 per cent sodium carbonate and 10 cc. of 10 per cent sodium hydroxide, and bake the residue at 130°C. for 3 hours. Remove the dry mass to the Palkin extractor with the least possible quantity of water (25–40 cc.) and add a little ether to prevent foaming and 10 cc. of a mixture of equal volumes of sulfuric acid and water. Extract the mixture 1 hour with ether, at the end of which time all the fumaric acid should be in the receiver. Heat the residue from the ether gently on the steam bath to expel most of the acetic acid and add 25 cc. of water, 3.5 grams of sodium chlorate, and 1 cc. of 1 per cent osmium tetra-oxide solution. Provide the flask with an air condenser and keep at about 50°C. overnight. After cooling, recover the osmium tetra-oxide by shaking with benzene, which dissolves it, and add 50 cc. of a solution of calcium acetate (16 grams of calcium acetate and 120 grams of acetic acid to the liter). Stir well, allow to stand about 3 hours, filter on a tared Gooch, dry to constant weight at 200°C<sup>1</sup>. and weigh as neutral calcium racemate. Calculate to the equivalent of malic acid.

TABLE 6.

*Determination of malic acid as calcium racemate in a fruit juice mixture.*

TAKEN				RECOVERED		
Juice	Tartaric	i-Malic	Citric	Tartaric	Calcium Racemate	Malic
cc.	gram	gram	gram	gram	gram	gram
23	0.10	0.10	0.25	0.08	0.1492	0.11
23	0.25	0.25	0.25	0.224	0.2693	0.20
25	none	none	none		0.0400	0.03
25	none	none	0.25		0.0793	0.056

The presence of citric acid, as shown by the last two experiments, interferes with the value of the method. Just what acid is formed from citric which gives the insoluble calcium salt remains to be determined.

Experiments with i-malic acid and with citric acid, run by the fumaric racemic acid method, except that instead of separating the racemic acid as calcium racemate it was precipitated as potassium acid racemate from 50 per cent alcoholic solution and titrated, gave the following results:

TAKEN	RECOVERED
0.25 gram i-malic acid . . . . .	12.87 cc. of 0.1 <i>N</i> sodium hydroxide, equal to 0.172 gram of malic acid.
0.50 gram citric acid . . . . .	00.00 cc. of 0.1 <i>N</i> sodium hydroxide.

<sup>1</sup> Later experiments showed that this higher temperature was necessary.

Operating in this manner, the citric acid does not give a precipitate as a neutral blank was obtained. Further development of the method will be undertaken, the separation and titration of potassium acid racemate being used in preference to removal of the citric acid as lead citrate by the Hartsen method.

In conclusion, the associate referee feels that the results are sufficiently encouraging to justify further work along these lines and so recommends to the committee<sup>1</sup>. It is expected that a method will be formulated to be submitted for collaborative work this year, and it is hoped that the results of such work may be presented at the next meeting of the association.

No report on canned foods was given by the referee.

*C. A. Browne:* It was my great pleasure to accompany Dr. Wiley to the 50th annual celebration of the founding of the Connecticut Agricultural Experiment Station, at New Haven, and in listening that day to the stories which Dr. Wiley and Dr. Jenkins told I learned more than I ever learned before. Dr. Wiley has just had his 81st birthday, and he not only attended the meeting which led to the founding of the association, but he has attended every one of the following meetings. This is the 41st. In presenting Dr. Wiley you will all join with me, I am sure, when I repeat the wish of an early secretary of the association, Clifford Richardson, "Long may he wave".

#### ADDRESS BY DR. WILEY.

MR. PRESIDENT, MEMBERS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, AND LADIES AND GENTLEMEN:

The only reason why Dr. Browne and most of you cannot emulate my example is because of the unfortunately late period at which you were born. To be eighty-one years of age, unless there be some recent discovery of aging men—like bleaching flour, all of a sudden—you have to be born early. Now, that was my great good fortune. If I had been consulted about the matter I should have taken the year 1844 for my birthday. I arrived on this planet at a time when everything was about to change. I do not know of a more opportune year to be born than the one I happened to choose. At that time the great problems that subsequently involved this country in a great internecine war were very

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 8: 85.

vigorously under consideration. My first recollection of anything happening on this terrestrial globe was when, with my parents, I went to Madison, Indiana, to see the soldiers returning from the Mexican War in 1848. At that time there was only one railway in the State of Indiana and that connected the city of Madison on the Ohio river with the city of Indianapolis. The soldiers that went to the Mexican war came down to Madison on the railway and embarked on the Ohio river for New Orleans; from there they were transferred to Mexico. They returned the same way. I remember very well, as the boats came around the bend near Hanover College, how I discovered the soldiers on the deck, and I remember also the shouting and waving of handkerchiefs in greeting.

After that came all the wonderful transformation due to the war between the North and the South. When I was a boy we called our neighbors to the south "rebels", but of course we do not do that now. As a result of that war we became a great free country. The blemish of slavery was wiped out.

Now, all these events that occurred in my early days account in some degree for the sentiments which I have, and they were a liberal education. I remember that my father was a poor farmer—I mean by that poor in the affairs of what we call wealth—on a very hilly farm near the Ohio river. I saw three separate farms slip off into the river, but we had a good subsoil and we dug down to another farm. In my first address, in Louisiana, I said: "My fellow citizens—I call you that because I am sure I am standing on my native soil".

That was a wonderful occasion to which Dr. Browne alluded, the celebration of the 50th anniversary of the founding of the Connecticut Agricultural Experiment Station. It was interesting in every way, and it leads me to the observation that we are getting along now when we can celebrate a golden jubilee. This is, I believe, the 41st meeting of this association. The first steps that were taken in regard to organizing agricultural chemists were taken at a meeting held in Cincinnati, in 1880. At that time a group of agriculturalists got together and talked things over. At the next meeting in Boston, the following year, a stronger and larger group got together and talked over the matter of organization among the agricultural chemists. In 1882 and '83, so far as I know, no further steps were taken, but in 1884 the Commissioner of Agriculture of Georgia invited agricultural chemists to meet and discuss matters relating to the welfare of agricultural industry.

As I got on the train in Washington—it was the first year after I came here—I met one of the leaders in agricultural chemistry, Prof. Johnson of Yale, for the first time. I went all the way to Atlanta with him and became very well acquainted. He was the first agricultural director at the Experiment Station at New Haven, and you all know his career. It was he who first realized the importance of joining agri-



cultural research with analytical work. I saw his first soil analysis at New Haven. It was a kind of mud advertised as a fertilizer which had been collected. His analysis, in his own handwriting, is still on exhibition at the Experiment Station. And there I saw the accuracy of his work and his exacting handwriting. They did not have typewriters in those days, and they have destroyed our ability to write and spell in these days. The real result was that the value of that soil was found to be about \$1.10 a ton. That shows that at the very beginning Prof. Johnson realized the necessity of protecting the Connecticut farmer from fraud so he could have more time to make wooden nutmegs. Prof. Johnson was the leader of agricultural chemists at that time.

I also had the great privilege soon thereafter of knowing Prof. Hilgard and learned to know him very well. I wish I could tell you how much I admired him. He was one of the earliest workers in agricultural research in this country. As Dr. Browne has said, the peculiarities of these workers were that they not only wanted to protect from fraud but they wanted to investigate. Hilgard was a born investigator. I knew him even after he retired from active work. He was a great man, one whom it was a great inspiration to know.

You may be of the opinion that any one connected with Harvard University is not a human being, but a celestial sort of an intellect. There is a poem that I happened to think of:

There is a town called Boston,  
The home of the bean and the cod,  
Where the Cabots speak only to Lowells,  
And the Lowells speak only to God.

And some one in New Haven wrote another:

There is a town called New Haven,  
The home of the truth and the light,  
Where God speaks to Jones in the very same tones  
As he does to Hadley and Dwight.

So, you are all wrong about the exclusiveness of the Harvard professors. They are really human beings when you get under their Bostonian cuticle. You probably remember the saying of some one at Yale: "He bit off more than he could chew", while the same sentiment would be expressed at Harvard as follows: "His incisors have severed more than his molars can masticate". Now, that is the principal difference between Harvard and Yale.

I have been interested in knowing many great workers. There was Prof. F. H. Storer, who was not so well known for his agricultural work as he was for his collaboration with Prof. Eliot in writing a splendid book on chemistry, but he did excellent work at the Bussey Institute. I also knew Brewer, at Yale, and he was just the counterpart of Johnson.

He was a fine, well-rounded man, almost as good-looking as I am. Johnson had dyspepsia, and you know what that means. But Brewer did a great work at Yale in agriculture, also, and he should not be forgotten when you speak of the early workers. Then, I knew Caldwell of Cornell, one of the pioneers in my field. And so it has been my privilege to know these men, and I esteem it a great privilege, one which you can never know, because I think there are so many great men now that we are lost in a great sea.

At Atlanta, at this meeting of which I have just spoken, it was agreed that we should have another meeting in Philadelphia in the autumn of that year, when the American Association for the Advancement of Science held its meeting there. In those days that association was also a powerful influence in this country. When I go to these meetings now, I see many that I never knew in the old days. I do not want this association to get larger than it is, because I do not know many of you now, and if I come to your meetings for the next 15 or 20 years, as I hope to, I won't know any of you. It was arranged to have this meeting in Philadelphia, and the only way we could have it was to hold it in a beer hall—that is, so as to have peace and comfort and—other things. And so we met there and organized this association. The name then selected has never been changed—Association of Official Agricultural Chemists.

So that is the way this association was formed. Now, let me say just a few words about what it has accomplished. If we go simply by statistics we may feel that we have not accomplished much in this association in the way of improving the fertility of the soil. It is true that the average crops which we are now harvesting are not much larger, according to the area cultivated, than they were when this association was first started 41 years ago, but there are some kinds of agriculture which have been immensely improved, and one is intensive agriculture. Those wanting to raise more on the same area have always appealed to the information which we have been able to give. Probably this appeal comes most frequently from the cotton-growing areas, because I doubt if much cotton is grown now without some help from this association; so far as corn and wheat are concerned there is not much help from the association. The reason is that there is not enough fertilizer to do this. But we have discovered by investigation many ways of obviating the commercial fertilizer, and this is particularly true in the case of nitrogen. We can grow leguminous crops and use them for fertilizers. For ages farmers have understood that if they followed clover with another crop they had a greater yield, but they did not know why. As nitrogen is the most expensive element in our fertilizers it seems to be the only natural one that can be generally employed, and the growth of leguminous crops may possibly so improve all our vast area of corn and wheat growing land that increased yields may be obtained. But if you will consult the pro-

ductiveness of particular crops, with the exception of cotton, you will find that they have not greatly increased in yield. Now we soon shall have recourse to the air for additional nitrogen.

But, what do they do? In intensive agriculture, in the growing of truck crops around the State, without these fertilizers and manures the truck farmer would be helpless. So, there you have a very vivid illustration of our utility. And the same is true of the phosphoric acid and the potash we use. We can't manufacture those, but Nature has done it for us. No country has been so blessed with the supply of phosphate rock as our own. There are large areas in this country of the finest kind of phosphate limestone. Take Florida, of which you may have heard. It has been described as being bounded on the east by the Volstead Act, on the west by Los Angeles, on the south by Cuba, and on the north by suckers. It is 200 miles by 400 miles by 4 feet, but most of it is covered with sand. Most unfortunately I had the opportunity, because of my early birth, of being the first person to make an agricultural survey of the Everglades. It was this way: Hamilton Disston, a rich manufacturer of saws, took an interest in that vast expanse. He had a vision which now is justified but which then was not. He determined to drain Lake Okechobee. This lake receives all the waters in Florida and then spreads out and just flows over a lot of saw-grass. His idea was to dig a great ditch to about where Palm Beach is now and let all this water run away. He made a proposition to the Legislature to do this and to receive in payment every other section of land. The Legislature agreed to this. He was so enthusiastic that he went before this body again and offered to pay \$1,000,000 for the State's half of the Everglades. That settled it. So he came to the Secretary of Agriculture and asked for help. The Secretary, even then, was always glad to get me out of town if he could, so he sent me down with Disston to survey the Everglades. We floated down the Kissimee river on a little steamboat called the "Hamilton Disston," went down backwards part of the way. I went around that lake, up tributaries and so on. At different places I took soundings of the soil, and at many places found 14 feet of vegetable matter. I found out that that soft phosphate-impregnated limestone underlies the whole State of Florida—and you can dig it out with a spade. Then it gets very hard and makes fine building stone, containing 2 or 3 per cent of phosphate. But this land is all covered by sand to the depth of 18 inches or more, and it is only in the hammock land, where there is the hard wood, that you have the real soil of Florida. That is where my land is—I am not boosting it. No one knows how much my land is worth now except those suckers. How are you going to get to that subsoil below? If you do get there it is below sea level. Well, that is impossible, and therefore all agriculture in Florida must depend upon our fertilizers. In the hill country of Florida where this subsoil comes

to the surface, you have it all at your disposal, and there is where the great agricultural areas of Florida will be. Of course citrus fruit and all that sort of thing can be grown on sand, but you can't grow vegetables on sand very well.

The State has already spent nine millions recovering the land, and it is not through yet, but the engineers have lowered the lake some 8 or 10 feet. Near the Caloosahatchie river where I took my first samples there is now a flourishing city, Moore Haven, and that land is often sold for a thousand dollars an acre just for growing vegetables. I said at that time: "You must be careful not to dry this out too much, because this soil contains about 25 per cent of resin due to the dropping pine leaves". Disston's own farm, which he recovered from Lake Tohopekaliga, got too dry and took fire, and they never could put it out, but the ashes are the most valuable in the State today. There is no other part of the world where the work that we are doing is going to be of so much value as in that very State after this boom has all died out, because there is something there. It seems to me that in the South our work ought to have greater appreciation, because the South depends more upon agricultural industries than does the North.

Now, I want to say this—that the spirit which has always animated this organization is one, I think, that is most admirable. While our work is largely regulatory—Dr. Browne has already illustrated that in the story he told about Johnson—nevertheless it is an important thing to protect the farmers from fraud, and that is one of the features that characterize our paternal government today. But it is no more paternalism than it is maternalism. As the mother is always the chief factor at home I should say that it is maternal. Well, a certain care like that is necessary because we can't always protect ourselves. We can only do it by the unity of the State. We must combine. Now, the next thing the farmer should do is to combine against all outside influences. If we are going to have unionism then the farmer will never come into his own until he joins the union. This is now being done in California and in Florida. Take, for example, my tangerines that are still growing. All I have to do is to grow them. I belong to the union. I don't have to pick them, pack them, or sell them. I just have to handle the checks. I went out to see my friend's tangerines growing—just 10 acres. They were a beautiful red color—when you see tangerines you "see red". His check for the ten-acre crop of tangerines was published in the local paper of the Brooksville Chamber of Commerce and he got for it, net, after paying all expenses, \$16,627.50. Now, can you beat that on any farm in the North. When you go to Florida, go to Brooksville.

It is always a great delight to me to meet you and your friends. It is something of a courtesy; it is something nice to an old gentleman who is on the verge of decay. You know, a few years ago, when I was travel-

ing around a great deal, a young lady on the train would always come to me and ask me what time it was. Now, she always goes to the blond young man behind me. I know that I am beginning to decay, but I do not feel that way. I feel just as full of life and vigor as I ever did. I am just as bull-headed, persistent, and unreasonable as I ever was. I always want to start a fight wherever I am—and I usually succeed. But I know that all this is to come to an end sometime, and I want you to know how much I appreciate you and the courtesy that you are showing me today, as your honorary president. I am always eager to meet you and I thank you most cordially for listening to me.

## TUESDAY—AFTERNOON SESSION.

### REPORT ON CEREAL FOODS.

By **RAYMOND HERTWIG** (Bureau of Chemistry, Washington, D. C.<sup>1</sup>),  
*Referee.*

The chapter on Cereal Foods in *Methods of Analysis* presents methods for the analysis of only three types of products—wheat flour, baked cereal products, and alimentary pastes—for this large and important class of foods. It is evident that future development of this chapter should include information on the analysis of other types of wheat products, as well as of the many products of the other cereals such as corn, rye, rice, barley, etc.

A cursory review of the present methods reveals their inadequacy in many instances to meet the needs of cereal analysts and investigators. The methods for wheat flour are deficient, and some of the existing methods require further study and development. The four methods for baked products are totally inadequate, and the methods for alimentary pastes, although quite up to date and complete, are also deficient.

The importance of the extension and perfection of these methods is fully recognized. The benefits will accrue to the entire field of agriculture and agricultural chemistry, to official investigational and regulatory work, and to routine work and research in the chemistry connected with the cereal and baking industries.

The referee recognizes that the accomplishment of this work is not a task for one man alone or even for a few men; it is an undertaking deserving the attention and concerted efforts of a large group of chemists and investigators.

Since wheat flour is the most important cereal product, the referee stressed the study of methods for its examination. In these studies he felt the urgent need of associates who would individually take upon themselves the study of new methods needed by the association, or the study and further development of existing methods to bring them abreast with recent progress in cereal chemistry. He made an effort to obtain the services of prominent chemists and investigators who are directly interested in these methods in order to assure the greatest mutual benefits to the association and the associates themselves. Those who were solicited and who so willingly offered their time and energy deserve the gratitude of the association.

Since the last meeting seven new associate referees were designated to study methods of analysis of flour and bread. Some of these appointments were made rather late in the year, which was a handicap to

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<sup>1</sup> Present address: Hecker-H-O Co., Inc., Buffalo, N. Y.

progress in the respective assignments. The newly appointed associate referees and the studies assigned them follow:

Methods for Sampling Flour: H. Runkel, Bureau of Chemistry, Minneapolis, Minn.  
Glutenin in Flour: M. J. Blish, University of Nebraska, to replace P. F. Sharp, resigned.

Gluten in Flour: C. B. Kress, Sperry Flour Mills, Stockton, Calif.

Hydrogen-ion Concentration of Flour: C. H. Bailey, University of Minnesota, Minneapolis, Minn.

Diastatic Value of Flour: C. O. Swanson, Kansas State Agricultural College, Manhattan, Kans.

Fat (Acid Hydrolysis Method), Lipoids, Lipoid Phosphoric Acid ( $P_2O_5$ ), Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent Alcohol, and Unsaponifiable Matter in Flour: Samuel Alfend, Bureau of Chemistry, St. Louis, Mo.

Methods for the Examination of Bread: L. H. Bailey, Bureau of Chemistry, Washington, D. C.

The referee was unable to obtain the services of an associate to study methods for starch in flour, but it is hoped that this refereeship may be filled for the coming year<sup>1</sup>.

The studies of the following assignments were continued by the same associate referees of the preceding year:

Moisture in Flour: G. C. Spencer, Bureau of Chemistry, Washington, D. C.

Ash in Flour: C. E. Mangels, North Dakota Agricultural College, Agricultural College, N. Dak.

Chlorine in Bleached Flour: Armin Seidenberg, Department of Health, New York City.

#### REPORTING RESULTS OF ANALYSES.

It is customary among chemists to report the analytical results of cereal foods, as well as of most other foods, on the basis only of the original sample as analyzed. Since the moisture content varies considerably for different lots and samples of the same kind of foods and since the moisture content of a lot or sample may vary from day to day, it is evident that in order to be definite and informative analytical data must be correlated with the corresponding moisture content of the product at the time of analysis. The analyses of the same product associated with different quantities of water give rise to confusion and misunderstandings in trade transactions if they are considered apart from the corresponding percentages of water. Hence the influence of the variations of the moisture content upon the percentage composition of the non-water ingredients—which are those of economic value—must be regarded in all considerations involving the composition and analysis of foods.

All analytical results of cereal foods should be reported on the basis of the original sample as analyzed. In addition, it is frequently advantageous to have the results given on some other basis prescribed by the

<sup>1</sup> *This Journal*, 1926, 9: 7.

circumstances of the application of the respective analyses. Certain circumstances require the expression of results on the basis of the total solids of the sample, while other circumstances require results on some arbitrary, conventional, uniform basis that makes for simplicity and convenience of understanding and interpretation. Therefore it is deemed advisable that the association methods for cereal foods prescribe the necessary directions for reporting results for each product for which methods of analysis are provided, in order to further the adoption of a universal system. In this connection, it can be prescribed in a general way that results be reported on at least two of the three following bases:

- (1) Original sample as analyzed.
- (2) Total solids in the sample.
- (3) Some accepted conventional uniform basis most favorable to the circumstances of application of the results.

#### METHODS FOR SAMPLING FLOUR.

The development of a standard method for sampling flour in storage is peculiarly difficult because it must be consistent with the fundamental theoretical principles of sampling as well as with those actual circumstances of collecting a practically representative sample from flour stored under a variety of conditions.

The scheme adopted by H. Runkel, the associate referee, for studying sampling methods is worthy of note. He organized a committee of one official and three unofficial cereal chemists to assist him in obtaining a proper perspective of the various factors of flour sampling. With the assistance of this committee and with information gained from a variety of sources in the trade, he was able to formulate a most satisfactory tentative method. He also suggests a plan of study necessary to bring the method to the standing of an official method.

#### TOTAL SOLIDS AND MOISTURE IN FLOUR.

The usual methods for the determination of moisture in flour are indirect methods that determine the loss in weight occurring under certain more or less definite conditions of pressure and temperature and define this loss as moisture. The loss in reality represents all volatile substances given off under the conditions of the determination. With flour this loss, beyond question, is essentially water, but with many other cereal foods these same methods determine appreciable quantities of non-water volatile substances in addition to the moisture. Fresh breads, for example, may contain 1-1.5 per cent alcohol and volatile acids. These volatile substances would be reported as water by the so-called moisture methods.

To avoid possible ambiguity in the application of the indirect moisture methods for flour and other cereal foods, they should be designated as



"indirect methods". It is believed that the title "total solids and moisture (indirect method)" would be appropriate.

G. C. Spencer, the associate referee, directed a careful collaborative study of the umpire vacuum method and the routine air-oven method for the indirect determination of moisture in flour. The collaborative results obtained by him clearly justify the recommendations he makes that these methods be adopted as official (first reading).

It is not out of place at this time to restate the reasons for proposing two methods for one determination.

The umpire vacuum method carefully defines those conditions of temperature and pressure for practically taking off the maximum quantity of water associated with flour. It will give results that can be more easily duplicated by analysts than any other method; it also defines the procedure for obtaining the most accurate results. This method standardizes the determination of total solids, and of moisture indirectly, so completely that it is believed to be the most appropriate and accurate method known for the purpose of its application. It is therefore denominated the "umpire" method.

The routine air-oven method is recommended for the same determination because it accomplishes practically the same results as the umpire method in a simpler and more economical manner and in shorter time. The conditions of temperature and atmospheric pressure stipulated in the method have no significance in themselves. The reliability of the results rests solely upon the fact that careful and extended investigation has shown that this method gives results very similar to those of the umpire method.

With the adoption of these two methods, the association will meet the needs of the investigator and also the needs of the practical routine chemist.

#### ASH IN FLOUR.

The collaborative results submitted by the Associate Referee on Ash in Flour did not justify a recommendation for the adoption of any of the three methods studied.

It is a question whether as a matter of policy the association should adopt a rapid method for ash in flour that changes the nature of the resultant ash such as occurs with two of the methods studied. The proposed calcium acetate methods very probably yield an ash material of radically different composition from that of the official method. The added calcium salt undoubtedly holds elements of an acid character that otherwise would be volatilized. The high temperature of ignition, namely 900°C. or above, also may be presumed to drive off to a greater or less extent some ash material non-volatile at the temperature of ignition of the official method, namely 550°C. These two factors may

by chance counterbalance in many instances and result in an apparent agreement with the official method. Therefore, it is a matter for consideration at this time not only whether the association will adopt methods such as the calcium acetate ash methods, but also whether it should even undertake the study of such methods. The Associate Referee on Ash for the coming year should investigate the attitude of the association on this matter before devoting time and energy to methods unacceptable to the association.

The associate referee recommends further study of the use of alundum in a rapid ash method. It is probable that alundum is inert chemically toward the flour ash constituents, and if so this type of method would not be open to the objections referred to previously. He also recommends discontinuance of study of the glycerol ash method. In this connection it is believed the incoming associate referee should investigate rapid ash methods which produce an ash of the same composition as that obtained by the official method.

**METHODS FOR FAT (ACID HYDROLYSIS METHOD), LIPOIDS, LIPOID PHOSPHORIC ACID ( $P_2O_5$ ), AND WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL, IN FLOUR.**

These methods are tentative for the analysis of alimentary pastes. Because they are of value to official chemists for the examination of flour and because it was believed that cereal chemists will find them applicable for flour analysis they were studied collaboratively under the direction of Associate Referee Samuel Alfend.

The collaborative results reported for all three methods are excellent. The associate referee recommends their adoption as official (first reading) in the form as given in his report, which differs in some minor alterations of technique and wording from the present tentative methods as adapted to alimentary pastes. Additional recommendations for a few minor changes in wording of the methods are made in the final recommendations of this report.

**METHOD FOR ORGANIC AND AMMONIACAL NITROGEN.**

Cereal chemists are ever in search of more rapid methods to increase their working efficiency and this is also believed to be a proper function of the association, but accuracy cannot be sacrificed for speed. At this time rapid methods are being considered for moisture and ash routine methods. Therefore, attention may be given profitably to the development of a rapid method for organic and ammoniacal nitrogen, since this method is so extensively used in cereal routine analyses.

**METHODS FOR GLUTENIN IN WHEAT FLOUR.**

The associate referee, P. F. Sharp, appointed in 1924 to study methods for glutenin, resigned early in the year because he had accepted a posi-

tion requiring his attention on an entirely different line of research. The association was fortunate in obtaining M. J. Blish of the University of Nebraska to take over these studies. Blish undertook a very thorough critical study of a direct method for glutenin proposed by himself and R. M. Sandstedt<sup>1</sup>. This was the only direct method for this protein known at the time of its publication. The studies consisted of notations on the effect of varying the details of the method on the results obtained. The associate referee states in his report that the quantitative accuracy of the method is not yet definitely established and outlines in detail the future work necessary before the method can be applied with assurance to glutenin determination.

#### GLUTEN IN FLOUR.

It is known that the present tentative quantitative method for gluten is not satisfactory and that determinations on the same flour by different laboratories may differ widely. Since many cereal chemists consider this determination of real value in flour analysis and certain investigations of Dill and Alsberg<sup>2</sup> indicate possible developments of the method, the association designated C. B. Kress, who already has reported investigations on gluten quality, as associate referee to study means of developing the method to the highest degree of perfection attainable. Owing to the late appointment of this associate referee, he has submitted no formal report.

#### METHOD FOR HYDROGEN-ION CONCENTRATION OF FLOUR.

C. H. Bailey, the associate referee to study methods for hydrogen-ion concentration of flour, submitted three samples of flours to a number of collaborators, requesting that they be analyzed for hydrogen-ion concentration according to the methods used in their respective laboratories and that the details of the procedure followed be reported with the results.

It is concluded from the collaborative results that the procedure for the preparation of the water extract for subsequent determination of hydrogen-ion concentration can be readily standardized. Other details reported will enable the associate referee to submit a definite method for this determination to collaborative study next year.

#### DIASTATIC VALUE OF FLOUR.

The associate referee, C. O. Swanson, assigned the study of the diastatic value of flour was unable to make any progress on these studies owing to his late designation and to the serious illness of a member of his department. No report was made.

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<sup>1</sup> *Cereal Chemistry*, 1925, 2: 57.

<sup>2</sup> *Ibid.*, 1924, 1: 222.

## METHOD FOR CHLORINE IN BLEACHED FLOUR.

The associate referee, Armin Seidenberg, continued his studies of a method proposed by him in 1924<sup>1</sup>. The method was designed originally to give negative results for flours untreated with chlorine, and positive results for chlorine-treated flours. The method was applied to eight untreated flours of different types with practically negative results in each instance. Two samples each of chlorine-treated patent and clear flours yielded decidedly positive results by the method. Insufficient time prevented the collaborative study of the method, but it should be submitted to such study next year.

## ACIDITY OF WATER EXTRACT OF FLOUR.

The present tentative method for determining the acidity of water extract of flour directs that the acidity be reported in terms of lactic acid. This manner of reporting results is unfortunate because the titratable acidity is not, at least in major quantity or possibly at all, due to lactic acid. It is believed that the most acceptable manner of measuring the titratable acidity of flours, which at the same time conforms to fact, is to express the results as cubic centimeters of 0.1 *N* alkali per 100 grams of sample.

The procedure for water extraction of the sample and for the subsequent filtration as directed by the method may be greatly simplified, and it should be studied with these modifications in mind.

## EXPERIMENTAL BAKING TEST OF FLOUR.

Although the experimental baking test is the most important method for judging the baking qualities of a flour it has not been standardized to meet the requirements of baking chemists. It is conceded in this connection that no straight-jacket method can be adopted for all the types of flours and all the purposes for which baking tests are used, but on the other hand it is believed that a considerable part of this method can be standardized in such fashion as to aid greatly the cereal and baking chemist. The association can render one of its most important services in the field of cereal and baking chemistry by sponsoring the study and development of a generally applicable method for making the experimental baking test or tests.

## METHODS FOR BREAD ANALYSIS.

The associate referee, L. H. Bailey, studied collaboratively an umpire vacuum method and a routine air oven method for the total solids of an entire loaf of bread, and also the comparative determination of the lipoids of bread by two methods, the present tentative method for fat

<sup>1</sup> *This Journal*, 1925, 8. 676.

in bread and the method for lipoids as given for alimentary pastes. He reports satisfactory results for the umpire vacuum method for the total solids of bread. The results for the routine method do not agree with those of the umpire method as closely as is desired. Insufficient results were obtained for the methods for fat and lipoids to permit the drawing of definite conclusions.

## METHODS FOR ALIMENTARY PASTE.

### PREPARATION OF SAMPLE.

The tentative method for the preparation of sample of an alimentary paste for analysis directs that 300–500 grams be ground “in a mill until all the material passes through a 60-mesh sieve”. This stipulation was written into the method because it was believed that a finely ground sample would favor a more complete extraction of lipoids, lipid  $P_2O_5$ , and water-soluble protein-nitrogen precipitable by 40 per cent alcohol than a coarsely ground sample. However, reduction of a hard, vitreous material like an alimentary paste to a powder that passes a 60-mesh sieve requires considerable time and labor. Since the reduction to pass a 20-mesh sieve would materially simplify the procedure for the prepara-

TABLE 1.

*Analysis of noodles of 20- and 60-mesh granulations.*

ANALYST	SAMPLE	DEGREE OF GRANU- LATION	MOISTURE (INDIRECT METHOD) VACUUM METHOD	LIPOIDS	LIPOID $P_2O_5$	WATER- SOLUBLE PROTEIN- NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
L. H. Bailey*	Whole-egg noodle	20-mesh	9.12	4.95	0.070	0.191
			9.12	4.98	0.070	0.196
R. Hertwig				5.01 5.06 5.07	0.075 0.079 0.079	0.199 0.207
L. H. Bailey		60-mesh	9.09	4.88	0.070	0.196
			9.09	4.96	0.071	0.207
R. Hertwig				4.98 5.01 5.04	0.075 0.078 0.079	0.204 0.207
L. H. Bailey	Yolk noodle	20-mesh		5.90 5.92	0.072 0.072	0.120 0.120 0.122
L. H. Bailey				5.77 5.92	0.072 0.072	0.109 0.109 0.112

\* Food Control Laboratory, Bureau of Chemistry.

tion of sample, some experiments were made by the referee to ascertain whether the coarser grinding affects the results obtained by the methods mentioned.

A sample of whole egg noodle and of a yolk noodle were ground very coarsely and well mixed to assure homogeneity. Each sample was then divided into two portions; one portion was reduced just to pass a 20-mesh sieve and the second portion a 60-mesh sieve. The sub-samples of each noodle representing 20-mesh and 60-mesh granulations were analyzed, and the results shown in Table 1 were obtained.

Since the results in Table 1 show that a 20-mesh sample of an alimentary paste analyzes the same as a 60-mesh sample by the methods for lipoids, lipid  $P_2O_5$ , and water-soluble protein-nitrogen precipitable by 40 per cent alcohol, it is necessary to grind such samples for the purposes of these determinations only fine enough to pass a 20-mesh sieve.

#### MOISTURE IN ORIGINAL UNGROUND SAMPLE.

The fineness of granulation of the sample demands attention from another standpoint in the determination of the moisture of the original unground material of an alimentary paste. The present tentative method determines the moisture of the prepared ground sample only and does not take into account the moisture changes incident to the grinding and sifting to pass a 60-mesh sieve. Since the moisture of an alimentary paste in its original condition is important in official regulatory work, L. H. Bailey of the Bureau of Chemistry and the referee carried out some experiments to provide a practical procedure for this determination.

A 5 pound sample of macaroni was broken into one inch fragments, mixed, and sealed in Mason jars with rubber gaskets. Three 400 gram portions were ground to pass 20-, 40-, and 60-mesh sieves, respectively. So far as possible the operations were made quantitative, and the ground portions were weighed. The total solids of each ground sample were determined by the umpire vacuum method for flour. The total solids for the original unground portions were calculated to include the losses incidental to grinding and oven drying.

The results are given in Table 2.

TABLE 2.  
*Total solids of 20-, 40-, and 60-mesh samples of a macaroni.*

GRANULATION	ORIGINAL WEIGHT	WEIGHT OF GROUND PORTION	TOTAL SOLIDS OF GROUND PORTIONS. (UMPIRE VACUUM METHOD—AVERAGES OF 3 DETERMINATIONS)		CALCULATED TOTAL SOLIDS OF ORIGINAL UNGROUND PORTIONS	
			<i>per cent</i> (1)	<i>per cent</i> (2)	<i>per cent</i> (1)	<i>per cent</i> (2)
20-mesh	400.0	398.5	88.19	88.23	87.86	87.93
40-mesh	400.0	397.9	88.13	88.17	87.67	87.71
60-mesh	400.0	396.0	88.27	88.29	87.39	87.41

From the results given in Table 2 it is apparent, as is to be expected, that the losses increased with finer grinding during the reductions of the three portions. The different results for the calculated total solids of the three unground portions may have two explanations: (1) The volatile substances determined by the vacuum method are not driven off as completely in the coarser as in the finer samples; (2) some of the losses incidental to the preparation of the finer samples are solid material, not moisture, and constitute the larger experimental error of reducing a sample to pass a 60-mesh than a 20-mesh sieve. It is believed to be impractical to prove definitely which of the preceding explanations is the more correct.

A method recently published by Bidwell and Sterling<sup>1</sup> for the direct determination of moisture was thought to offer possibilities for the direct determination of moisture in alimentary paste and consequently was given a trial. Results by this method are given in Table 3. For comparison those of the preceding indirect method are also given.

TABLE 3.  
*Moisture in alimentary paste by direct method\*.*

GRANULATION	ORIGINAL WEIGHT	WEIGHT OF GROUND PORTION	MOISTURE, DIRECT METHOD	CALCULATED TOTAL MOISTURE BY DIRECT METHOD OF ORIGINAL UNGROUND PORTION	CALCULATED TOTAL MOISTURE OF ORIGINAL UNGROUND SAMPLE BY INDIRECT METHOD FROM TABLE 2
20-mesh	grams 400.0	grams 399.2	per cent 11.35† 11.30‡	per cent 11.53 11.48	per cent 12.14
1 inch pieces			7.50†		
1 inch pieces			9.75‡		

\* Analyses by W. F. Sterling, Bureau of Chemistry.

† Distilled with toluol.

‡ Distilled with xylol.

The results given in Table 3 clearly show that this direct method for moisture is not applicable to alimentary paste.

A sample of an egg noodle of rather high moisture content was analyzed for total solids of the original material according to the most favorable procedure ascertained by the preceding experiments. The sample was broken up into small fragments and mixed. Two 500 gram portions were ground to pass a 20-mesh sieve. The high fat and moisture content caused the sample to lump and prevented the preparation of a third portion to pass a 40-mesh sieve. The prepared sub-samples were analyzed for total solids by the umpire vacuum method proposed for flour.

Several points may be gained from the experiments with the moist sample of noodles. Only slight loss occurs if the sample is reduced to pass only a 20-mesh sieve. Samples that are rather moist and oily

TABLE 4.  
*Total solids of noodle sample.*

GRANULATION	WEIGHT OF SUB-SAMPLE	WEIGHT OF POWDERED SUB-SAMPLE	TOTAL SOLIDS OF PREPARED SUB-SAMPLE			CALCULATED TOTAL SOLIDS OF ORIGINAL UNGROUND MATERIAL		
			(1)	(2)	(3)			
			UMPIRE VACUUM METHOD					
			Dried 5 hrs.	Dried 11 hrs.	Dried 30 hrs.	(1)	(2)	(3)
20-mesh	grams 500.0	grams 498.0	per cent 85.78 85.80	per cent 85.62 85.62	per cent 85.54 85.46	per cent 85.45	per cent 85.28	per cent 85.16
20-mesh	500.0	499.8	....	....	85.26 85.24			85.22

cannot be run through sieves of 40-mesh or finer because of lumping. A slight loss in the vacuum drying of the ground sample occurs after 5 hours' heating, but this loss is so inconsequential that it may be disregarded for practical purposes.

As a result of the preceding studies and discussions it is believed that the tentative method for the preparation of sample of alimentary pastes should be modified to the extent of directing that 400-500 grams of the sample be ground until all the material passes through a 20-mesh instead of a 60-mesh sieve. Also the tentative method for moisture should be replaced by the umpire vacuum method for total solids and the indirect determination of moisture as recommended for flour, and provision should be made for the estimation of the total solids or moisture of the original unground material.

#### METHOD FOR WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

The tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in alimentary pastes directs that the mixture of precipitated protein, alcohol, and asbestos be filtered through a mat of asbestos in a Hirsch funnel and light suction used. The filtration is quite rapid for small quantities of precipitated protein, such as are obtained from flour, plain alimentary pastes, and pastes of rather low whole-egg content, but it becomes increasingly difficult for large quantities of precipitate from noodles of 5-8 per cent whole-egg content. The referee found that these difficulties of filtration can be overcome by centrifugalization, which firmly packs the precipitated protein and asbestos mixture in the bottom of the centrifuge tube and permits the decantation of the clear supernatant liquid. By this means the separation and washing of large quantities of the precipitated protein are



rapidly performed. This procedure was found so satisfactory that a recommendation is made in this report that the method be revised to include these operations in detail.

#### TEST TO DISTINGUISH SWEET CORN FROM FIELD CORN.

During the past year several members of the Bureau of Chemistry have perfected a test to distinguish between sweet and field corn. The test is of especial interest in official regulatory work. The method should be put into shape for collaborative study and if satisfactory should be included with the regular cereal food methods. It may be mentioned in this connection that many valuable methods and tests that are devised from time to time to meet particular needs do not find their way to permanent records and frequently become lost in the mass of published chemical data. Methods that have real merit should be adopted by the association.

#### METHODS FOR THE ANALYSIS OF WHOLE GRAIN.

*Methods of Analysis* contains no methods specifically for the analyses of whole grains, such as wheat, corn, oats, barley, rye, kaffir corn, etc., although they are essential and important to agricultural chemists and investigators, to the cereal industry, and to official chemists. The referee suggests that the association designate an associate referee for the study of the most important methods for the examination of such whole grains. This work should include the collaborative study of the methods and their preparation in a form for adoption. An effort should also be made to harmonize the methods for these products with the association methods for cereal foods that are of most recent development or with those methods now being studied.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the following directions for the reporting of results of analyses be inserted in the next revision of *Methods of Analysis* immediately under each respective heading designating the product for which the subjoined methods are provided:

(a) Under "Wheat Flour".

Report results on at least two of the three following bases:

- (1) Original sample.
- (2) Total solids in the sample.
- (3) 13.5 per cent moisture in sample.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 88.

## (b) Under "Bread".

Report results on at least two of the four following bases:

- (1) Original entire loaf.
- (2) Total solids in the original entire loaf.
- (3) Original entire loaf of 62 per cent total solids.
- (4) Air-dried sample.

## (c) Under "Alimentary Pastes".

Report results on at least two of the three following bases:

- (1) Original unground sample.
- (2) Prepared ground sample.
- (3) Total solids in the sample.

(2) That the method for sampling flour as given in the report of the associate referee be adopted as tentative and that steps be taken during the coming year to prepare the method for adoption as official. The future studies should include the independent sampling by this method of some commercial lot of flour by a number of analysts and the comparison of their results. Official and commercial cereal chemists should be solicited for these collaborative studies.

(3) That the umpire vacuum method for the determination of total solids and moisture (indirect method) in flour be adopted as official (first reading). (This method has been published under the heading "Vacuum Method—Tentative".<sup>1</sup>)

(4) That the routine air-oven method for the determination of total solids and moisture (indirect method) in flour be adopted as official (first reading). This method has been published<sup>2</sup>.

(5) That the Associate Referee on Ash in Flour investigate any possible rapid ash methods that yield an ash of the same composition as the official method. Should he deem it advisable to propose a method that produces an ash of different composition than the latter method but of possible equal percentage, he should obtain beforehand the opinion of the association as to the acceptability of the principles of the method.

(6) That the methods for the determination of fat (acid hydrolysis method), lipoids, lipoid phosphoric acid ( $P_2O_5$ ), and water-soluble protein-nitrogen precipitable by 40 per cent alcohol, in flour, as described by the associate referee in his report, be adopted as official (first reading) with the following changes:

(1) Method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol:

- (a) Delete "if not available, shake by hand".
- (b) Change "0.1 gram asbestos" to "0.1–0.3 gram asbestos".
- (c) Change "in a Gooch crucible" to "in a Gooch crucible or Hirsch funnel".

(2) Method for fat (acid hydrolysis method):

- (a) Change "immerse" to "set".
- (b) Change "at frequent intervals for 30–40 minutes" to "at frequent intervals for 30–40 minutes, or until the proteins and starch are sufficiently hydrolyzed to form a clear solution".

<sup>1</sup> *This Journal*, 1926, 9: 39.

<sup>2</sup> *Ibid.*, 40.

- (c) Change "ether previously washed with water" to "ether".
  - (d) Add the following paragraph to the method: "Higher results are obtained by this method than by direct ether extraction. The fat determined probably consists essentially of the true fats, fatty acids, unsaponifiable matter, and coloring matter. Such substances as lecithin are largely destroyed by the acid hydrolysis".
- (3) Method for lipoids and lipoid phosphoric acid ( $P_2O_5$ ):
- (a) Change the second sentence in part to read: "all the particles with the alcohol, stopper and set . . . etc.".
  - (b) Immediately preceding the second last sentence add: "Wash the residue and filter well with hot water".
  - (c) Add the following paragraph to the method: "The extracted lipoids contain practically all the ether-soluble substances in flour, true fats, fatty acids, lecithin and allied substances, unsaponifiable matter, waxes, coloring matter, etc. This method extracts more ether-soluble substances than direct ether extraction".

(7) That an associate referee be designated to study rapid methods for the determination of organic and ammoniacal nitrogen in flour with a view to the adoption of a routine method that will yield results closely approximating those of the official method.

(8) That the study of the method for glutenin in flour be continued as indicated in the report of the associate referee.

(9) That study of methods for the determination of gluten in flour be continued during the coming year.

(10) That study of methods for the determination of the hydrogen-ion concentration of flour be continued according to the recommendations of the associate referee.

(11) That study of methods for the determination of the diastatic value of flour be continued during the coming year.

(12) That the method for the determination of chlorine in chlorine-treated flours proposed by the associate referee be studied collaboratively during the coming year.

(13) That the tentative method for the determination of the acidity of water extract of flour be studied with a view to the simplification of the extraction and filtration and the reporting of the results other than as lactic acid.

(14) That an associate referee be designated to study methods for the determination of starch in flour.

(15) That methods for the determination of unsaponifiable matter in flour and alimentary paste be studied during the coming year.

(16) That one or more associate referees be designated to study methods for the examination of whole grain, as wheat, corn, oats, barley, rye, etc. Where possible these methods should harmonize with the association methods for cereal foods that are of recent development or are being actively studied.

(17) That an associate referee be designated to study the test devised by analysts of the Bureau of Chemistry to distinguish between field and sweet corn.

(18) That the sub-heading "Bread" be placed immediately under the heading "Baked Cereal Products" in the chapter on Cereal Foods in the next revision of *Methods of Analysis*.

(19) That the Hertwig-Bailey method<sup>1</sup> for the determination of the total solids of an entire loaf of bread be adopted as official (first reading).

(20) That the tentative method for preparation of sample of bread be adopted as official (first reading) and be published in the next revision of *Methods of Analysis* immediately following the method for total solids of an entire loaf of bread. This method has been published<sup>1</sup>.

(21) That the method for the determination of the total solids of the air-dried ground sample prepared as directed under Recommendation 20 be adopted as official (first reading). This method has been published<sup>1</sup>.

(22) That the tentative method for the determination of ash in bread be adopted as official (first reading). This method has been published<sup>1</sup>.

(23) That the tentative method for the determination of protein in bread be adopted as official (first reading). This method has been published<sup>1</sup>.

(24) That a routine air-oven method for the determination of the total solids of an entire loaf of bread be further studied. This study should include the use of a temperature of 130°C., such as is specified in the routine method for total solids in flour.

(25) That further comparative study be made of the methods for the determination of lipoids (as directed for alimentary pastes) and fat (tentative for bread) in bread.

(26) That another method for collection and preparation of sample of alimentary pastes be adopted as official (first reading) to replace the present tentative method. This method has been published<sup>2</sup>.

(27) That another method for the determination of total solids and moisture (indirect method) in alimentary pastes be adopted as official (first reading) to replace the present tentative method for moisture. This method has been published<sup>2</sup>.

(28) That the tentative methods for the determination of ash<sup>3</sup>, chlorides in ash as sodium chloride, organic and ammoniacal nitrogen, and extraction and identification of added color in alimentary pastes be adopted as official (first reading).

(29) That the method for the determination of protein in alimentary paste<sup>4</sup> be adopted as official (first reading) and be given in the next revision.

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<sup>1</sup> *This Journal*, 1926, 9: 42.

<sup>2</sup> *Ibid.*, 43.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 232, 233.

<sup>4</sup> *This Journal*, 1926, 9: 44.

sion of *Methods of Analysis* immediately after the method for organic and ammoniacal nitrogen.

(30) That the tentative methods for the determination of fat (acid hydrolysis method), lipoids, lipid phosphoric acid ( $P_2O_5$ ), and water-soluble protein-nitrogen precipitable by 40 per cent alcohol in alimentary pastes be modified to agree with the respective methods of Recommendation 6 for flour in this report and adopted as official (first reading). The following additional sentence should be added to the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol and just preceding the sentence "Allow to stand overnight": "Use 50 cc. of the filtrate instead of 100 cc. with egg pastes containing large quantities of albumin".

(31) That the following paragraphs be added to the method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol in alimentary pastes:

It will be found convenient to separate and wash large quantities of the alcohol precipitated protein from the mother liquid by the aid of centrifugalization, for which proceed as follows:

Transfer the mixture of alcohol solution, the precipitate, and 0.2-0.5 gram of prepared, ignited asbestos to a centrifuge tube. (A satisfactory centrifuge tube, capacity about 125 cc., diameter about 4 cm., and length about 15 cm., is readily made from a heavy glass tube. The bottom of the tube for a length of 6-8 cm. is tapered down to a diameter of about 1 cm. at the sealed end. This tube should be cushioned in a rubber stopper that fits snugly in the trunnion cup to prevent breakage during centrifugalization.) Distribute the asbestos well with the precipitate. Whirl at a high speed until the precipitate and asbestos are packed firmly in the bottom of the tube. Decant the supernatant liquid through an asbestos mat in a Hirsch funnel or Gooch crucible, using light suction. Add about 20 cc. of 40 per cent alcohol to the tube, mix well with the precipitate with the aid of a glass rod, rinse off the rod with a little 40 per cent alcohol, centrifugalize and decant off the supernatant liquid as before, through the same filter. Wash medium quantities of precipitate twice in the above manner, and large quantities three times. Transfer the precipitate and asbestos to a Kjeldahl flask by means of a stream of hot water from a wash bottle. Use a rubber policeman to remove any precipitate adhering to the tube. Add the filter mat to the Kjeldahl flask and proceed as directed above.

(32) That study of the routine air-oven method for the determination of total solids in alimentary pastes be continued during the coming year.

(33) That an associate referee be designated to study methods for making the experimental baking test or tests of flour.

## REPORT ON MOISTURE IN FLOUR.

By G. C. SPENCER (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

All efforts this year were devoted to reviewing and confirming the two moisture methods that were presented to this convention a year ago<sup>1</sup>.

<sup>1</sup> *This Journal*, 1925, 8: 301.

The entire work here to be reported is collaborative in nature and is conveniently divided into two parts.

#### COLLABORATION WITH THE FOOD CONTROL LABORATORY OF THE BUREAU OF CHEMISTRY.

During a period of about six months, beginning in November 1924, the official inspectors were forwarding flour samples from representative mills all over the country to the Bureau of Chemistry in Washington where the moisture content of each sample was determined as soon as possible by the new, short method of drying at 130°C. for one hour. This work was done by chemists in the Food Control Laboratory and was repeated on 21 samples by the associate referee, who used both the vacuum or "umpire" method and the routine method which employs a temperature of 130°C. The Food Control chemists made their determinations in duplicate only, while the associate referee determined the moisture in quadruplicate by each method.

The results of this type of collaboration serve to demonstrate the applicability of these new methods to a large number of typical flour samples from widely separated sources.

#### DISCUSSION OF RESULTS.

In all cases the estimations of the associate referee were made several weeks later than those of the Food Control chemists.

In most cases the results by the vacuum method are lower than those obtained at 130°C.

#### COLLABORATION WITH INDIVIDUAL WORKERS ON THE SAME SAMPLE.

The sample of flour was prepared this year by mixing and sifting together a number of flour samples remaining after other tests had been completed. This uniform mixture was quickly sealed in glass fruit jars of one quart each and forwarded to the collaborators.

#### INSTRUCTIONS TO COLLABORATORS.

##### (a) *Vacuum or "Umpire" Method.*

Weigh accurately about 2 grams of sample in a tared, covered dish. Loosen the cover and heat the dish and contents in a vacuum oven to 98°–100°C. for 5 hours at a pressure of not more than 25 mm. (1 inch) of mercury. Tighten the cover on the dish and cool for 20 minutes in a desiccator. Weigh and calculate the loss in weight as moisture.

##### (b) *Routine Method.*

Weigh accurately about 2 grams of the sample in a tared, covered dish. Remove the cover and heat the dish and contents in air in an oven at 130°C. for 1 hour. Replace the cover on the dish and cool in a desiccator for 20 minutes. Weigh and calculate the loss in weight as moisture.

## CONDITIONS OF MAKING THE TESTS.

In each case make at least four determinations.

Aluminum dishes, cylindrical in shape, 18 mm. high and 60 mm. in diameter, with close fitting inside covers are recommended (A. H. Thomas & Co.'s Cat. No. 4521).

*Method A—Vacuum or "Umpire".*

## Drying Conditions:

Pressure.....	25 mm. or less
Temperature.....	98°–100°C.
Time.....	.5 hours
Dish.....	Loosely covered

*Method B—"Routine".*

## Drying Conditions:

Pressure.....	Atmospheric
Temperature.....	130°C.
Time.....	1 hour
Dish.....	Uncovered

*Method C—"Routine" 1st modification.*

Temperature.....	125°C.
Other conditions same as Method B.	

*Method D—"Routine" 2nd modification.*

Temperature.....	135°C.
Other conditions same as Method B.	

Fifteen samples were sent out between February 12 and April 16 to as many collaborators. Of these, twelve reported before September 1, and their complete results are expressed in Table 2.

On several occasions the question of temperature variation has been raised concerning the "routine" method. The collaborators, therefore, were requested to dry their samples at temperatures 5 degrees below and 5 degrees above the stipulated 130 degrees in order to obtain data that would furnish information on this point.

The results shown in Table 2 indicate that a temperature variation of 5 degrees or less will have no serious effect.

Attention is directed to the report of G. A. Shuey<sup>1</sup>, Referee on Moisture Methods for the American Association of Cereal Chemists. In this report Shuey announces the intention of studying the moisture methods of this association. He accordingly sent out flour samples to his collaborators with the same instructions that are recorded in this report.

The results obtained are satisfactory and serve to confirm the findings of the associate referee.

<sup>1</sup> *Cereal Chemistry*, 1925, 2: 318.

TABLE 1.

*Results of determinations of moisture in flour samples obtained by Food Control Laboratory and the Associate Referee.*

MILL NO.	LOCATION	FOOD CONTROL ROUTINE	ASSOCIATE REFEREE					
			Routine Method			Vacuum Method		
			Maximum	Minimum	Average	Maximum	Minimum	Average
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	California	13.69 13.60	13.52	13.43	13.49	13.53	13.45	13.49
2	Colorado	14.95 14.96 16.20 16.17	15.17 16.06	14.76 15.92	14.93 15.97	14.90 16.02	14.86 15.89	14.88 15.95
3	Georgia	14.72 14.68 14.45 14.43	14.72 14.21	14.51 14.05	14.61 14.14	14.62 14.15	14.57 14.12	14.60 14.13
4	Illinois	14.15 14.19	13.89	13.82	13.85	13.76	13.57	13.66
5	Louisiana	14.23 14.30	14.34	14.28	14.31	14.33	14.29	14.31
6	Maryland	14.58 14.53	14.37	14.30	14.34	14.34	14.30	14.32
7	Massachusetts	14.85 14.86 15.60 15.58	14.71 15.53	14.71 15.36	14.71 15.44	14.71 15.43	14.54 15.31	14.63 15.39
8	Minnesota	14.30 14.31 14.36 14.25 14.36 14.35	14.23 14.13	14.16 14.07	14.19 14.10	14.37 14.00	14.18 13.88	14.26 13.95
9	New Hampshire	15.88 15.90	15.65	15.53	15.57	15.62	15.42	15.53
10	New York	15.41 15.45	14.83	14.64	14.76	14.79	14.52	14.65
11	Illinois	15.04 14.96	14.77	14.69	14.72	14.81	14.75	14.78
12	Virginia	15.04 15.04	14.39	14.34	14.38	14.19	14.08	14.15
13	Texas	14.02 14.01	13.89	13.56	13.76	13.93	13.89	13.91
14	Pennsylvania	14.33 14.37	14.20	13.96	14.06	14.06	13.88	13.99
15	New York	14.77 14.81	14.92	14.74	14.84	14.78	14.70	14.74
16	Virginia	15.90 16.00	15.52	15.27	15.40	15.31	15.20	15.26
	Average of means	14.79			14.54			14.49



TABLE 2.  
Results of determinations of moisture in flour obtained by collaborators.  
(Expressed in percentage.)

NO.	DATE	COLLABORATOR	METHOD A—VACUUM			METHOD B—130°C.			METHOD C—125°C.			METHOD D—135°C.		
			Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age	Maxi- mum	Mini- mum	Aver- age
1	5/25	L. H. Bailey	12.94	12.92	12.93	13.04	13.00	13.02	12.94	12.88	12.91	13.00	12.98	13.00
2	3/13	M. J. Blish	13.06	13.03	13.05	12.96	12.87	12.91	12.91	12.89	12.90	12.98	12.96	12.97
3	3/28	M. M. Brooke	13.02	12.96	13.00	12.84	12.80	12.82	12.83	12.76	12.79	12.99	12.88	12.94
4	3/30	D. A. Coleman, No. 1						12.82			12.67			12.87
5	3/30	D. A. Coleman, No. 2						12.89			12.72			12.89
6	3/30	D. A. Coleman, No. 3						12.85			12.74			12.84
7	3/30	D. A. Coleman, No. 4						12.85			12.59			12.82
8	5/20	F. A. Collatz, No. 1	12.75	12.75	12.75	12.85	12.80	12.83	12.85	12.85	12.85	12.74	12.69	12.72
9	5/20	F. A. Collatz, No. 2	12.71	12.69	12.70	12.85	12.83	12.84	12.77	12.76	12.77	12.74	12.68	12.71
10	4/18	J. T. Flohill	12.92	12.88	12.89	12.80	12.60	12.70	12.73	12.52	12.64	12.95	12.80	12.87
11	5/1	B. R. Jacobs	12.98	12.91	12.94	12.71	12.53	12.63	12.62	12.58	12.60	12.95	12.87	12.91
12	6/29	C. B. Kress	13.02	12.80	12.94	12.50	12.35	12.39	12.30	12.10	12.17	12.78	12.45	12.59
13	2/25	A. W. Meyer	13.19	13.15	13.18	13.22	13.03	13.12	13.15	13.00	13.08	13.23	13.12	13.17
14	4/17	J. B. Mudge	12.83	12.66	12.78	13.07	12.94	12.99	13.07	12.79	12.89	13.08	12.87	12.92
15	2/27	G. C. Spencer	12.97	12.87	12.92	13.12	13.02	13.07						
16	7/9	W. C. Luckow	12.66	12.36	12.51	12.57	12.07	12.33	12.66	11.97	12.31	12.75	12.45	12.57
17	7/9	P. L. Gerber	12.59	12.42	12.50	12.38	12.32	12.35	12.42	12.33	12.38	12.41	12.36	12.39

## REPORT ON ASH IN FLOUR.

By C. E. MANGELS<sup>1</sup> (Agricultural Experiment Station, Fargo, N. Dak.),  
*Associate Referee.*

The official method for ash in flour as adopted last year provides for ignition in a muffle furnace at a temperature sufficiently low to prevent fusion of the ash. The procedure recommended last year was originally suggested by C. H. Bailey of Minnesota. When subjected to collaborative study by the associate referee, the method gave consistent and concordant results<sup>2</sup>.

A high degree of accuracy in determining the ash content of flour is essential, and at the 1924 convention of the American Association of Cereal Chemists the Methods Committee of the association recommended that a tolerance of only 0.02 of 1 per cent between results of different laboratories be considered allowable. The recommendation made was based on the opinion of leading cereal chemists, and the narrow tolerance indicates that the cereal chemists are maintaining a high degree of accuracy in ash determinations.

Accurate weighing of containers and proper control of temperature of ignition are the essential points for obtaining concordant results for ash content of flour. In this connection the associate referee wishes to call attention to the fact that the phraseology of the method for ash in flour, as printed in the new edition of *Methods of Analysis*<sup>3</sup>, differs materially from the procedure recommended by the associate referee and adopted by the association.

The method as recommended by the associate referee reads as follows:

Ignite a crucible, and when cooled, weigh, and rapidly weigh into it 5 grams of flour. Ignite in a muffle at approximately 550°C., taking care that no portion of the muffle becomes sufficiently hot to fuse the ash. A light gray fluffy ash should result. Cool crucible and contents in a desiccator and weigh immediately after it reaches the temperature of the laboratory air.

The method as printed in the new edition of *Methods of Analysis*, however, reads as follows:

Weigh 3-5 grams of the well mixed sample into a shallow, relatively broad ashing dish, which has been ignited, cooled in a desiccator, and weighed soon after attaining room temperature. Incinerate in a furnace at approximately 550°C. (dull red) until a light gray ash results or until no further loss in weight occurs. Cool in the desiccator and weigh soon after room temperature is attained.

Reignited quick lime or calcium carbide is a satisfactory drying agent for the desiccator.

The present method specifies "a shallow, relatively broad ashing dish". In the writer's opinion the use of this type of container gives no appre-

<sup>1</sup> Presented by C. B. Morison.

<sup>2</sup> *This Journal*, 1924, 8: 140; 1925, 8: 671.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 225.

chable advantage either in time or accuracy, but it is objectionable for two reasons: (1) a broad shallow dish takes up relatively more space in the muffle and thus cuts down the capacity of the furnace; and (2) when handling a shallow dish particles of ash are more likely to be blown out and lost.

The two words "dull red" have also been inserted after the temperature specification. From the phraseology it would be inferred that the temperature may be estimated by the color of the ignition chamber, but in the writer's experience such a method for the determination of temperature is very inaccurate. The majority of modern flour laboratories have muffles equipped with pyrometers because proper control of temperature in ashing flour is essential for consistent results.

In the method as recommended the chemist is particularly cautioned against allowing the ash to fuse (an important point), but this caution is omitted in the method as printed. The printed method also recommends the use of reignited quick lime and calcium carbide as desiccating agents. This specification, in the writer's opinion, is undesirable, because these desiccating agents are not in common use and their superiority over other desiccating agents for this purpose has not been proved.

The method for ash in flour, as recommended, has given concordant results when subjected to collaborative study. The objectionable feature is the time required to ash the sample. A more rapid method for ash in flour would be desirable, provided the method would give accurate results and also be suitable for routine work. During the past two years the writer has subjected a number of suggested rapid ash methods to collaborative study.

#### GLYCEROL METHOD.

The glycerol method for ash in flour was suggested by L. H. Bailey and R. Hertwig in 1924<sup>1</sup>. C. H. Bailey reported results of collaborative study of this method by the Committee on Methods of the American Association of Cereal Chemists<sup>2</sup>. Collaborative work on this method was repeated in 1924 by the writer as Associate Referee on Ash, and the results of these collaborative studies were presented in his report to the 1924 convention. Collaborative study was also made by J. C. Wood in 1925 for the Committee on Methods of the American Association of Cereal Chemists<sup>3</sup>.

Since such an extensive collaborative study of the glycerol method has been made further work on this method was deemed unnecessary by the associate referee. A summary of the results of this collaborative study follows:

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<sup>1</sup> *Cereal Chemistry*, 1924, 1: 82.

<sup>2</sup> *Ibid.*, 189.

<sup>3</sup> *Ibid.*, 1925, 2: 246.

## GOOD POINTS OF METHOD.

1. The glycerol method gave consistent and concordant results and closely checked the official method.
2. Some time is saved in igniting of sample, and final results may be secured more quickly.

## DISADVANTAGES OF METHOD.

1. The mixing of the glycerol solution with flour is a tedious operation which requires considerable time in case a number of samples are run at the same time.
2. Unless a large dish is used, there is danger of loss owing to the frothing of the glycerol flour mixture. To avoid this loss the sample must be carefully charred over a free flame—another tedious operation.
3. If a large container is used for ashing with the glycerol method, the frothing may be disregarded, but the use of the large dish cuts down the capacity of the muffle furnace and precludes the use of the method in laboratories where a number of samples must be run every day.
4. The time saved in igniting the sample in the opinion of collaborators did not compensate for the extra time required to prepare the samples. In this respect the method is not so desirable as the present official method for routine work.

Owing to the disadvantages cited and to the fact that the glycerol method does not give increased accuracy and is not so suitable for routine work as the present method, the associate referee recommends that study of this method be discontinued.

## NEW METHODS STUDIED.

The present year's work on ash consisted of a collaborative study of three suggested rapid methods for ash and their comparison with the official method.

One sample of patent flour and the following instructions were sent to collaborators:

*Method I—Official Method.*

Follow the official A. O. A. C. method, but use a 2 gram sample of flour. Ignite at 550°C. until a light gray ash is obtained. Record the time required.

*Method II.*

Weigh into a crucible or other ashing dish 4 grams of alundum. Ignite the crucible and alundum in the muffle and weigh. Weigh into the tared container 2 grams of flour. Prepare a paper stirring rod by tightly rolling a small triangular piece of ashless filter paper ( $\frac{1}{4}$  of a 11 cm. paper is very satisfactory), and with this rod carefully and thoroughly mix the flour and alundum. Leave the paper rod with the sample and ignite in a muffle at 550°C., taking care that no part of the ash fuses. (Care should be taken to prevent any loss of alundum by mixing and handling.)

*Method III—Calcium Acetate Method.*

Weigh into a tared ashing dish 2 grams of flour and carefully measure and add 3 cc. of 0.5 per cent calcium acetate solution (Solution A). Mix flour and acetate thoroughly with a glass stirring rod and wipe off the rod with a piece of ashless filter paper which is added to the sample. Dry in an electric oven at 100°C. and ignite in a muffle, bringing the temperature to 900°C. and keeping at this temperature or higher for 40 minutes. A white ash should result.

Prepare two blanks by measuring carefully 3 cc. of the acetate solution (A) into a tared container, add a piece of ashless filter paper approximately the same size as used in wiping the rod for sample, dry, and ignite. Correct for blanks when calculating the percentage of ash.

*Method IV—Calcium Acetate—Acetic Acid Method.*

## PREPARATION OF SOLUTION.

*Acetate solution.*—Prepare by dissolving 1 gram of C. P. calcium acetate in 100 cc. of warm glacial acetic acid. Add 1 cc. of water and filter into a 200 cc. volumetric flask. Wash the filter with glacial acetic acid and finally make up to volume of 200 cc. with glacial acetic acid (Solution B).

## DETERMINATION.

Weigh into a tared container 2 grams of flour and carefully measure and add 3 cc. of the calcium acetate-acetic acid solution. Prepare a paper stirring rod by tightly rolling a small triangular piece of ashless filter paper and by punching and stirring thoroughly mix the acetate-acetic acid solution with the flour sample. Prepare two blanks in the same manner, using the same sized piece of filter paper in the blank. Start the ignition in a cool muffle, finally bring the temperature to 900°C., and keep at this point or higher for 40 minutes. Correct for blanks when calculating the percentage of ash.

NOTE: Alundum and Solutions A and B are furnished with sample. Solution A is for Method III and Solution B for Method IV. Results should be reported on blanks furnished.

No type or size of dish is specified, and the collaborators are requested to use dishes commonly used in their laboratories and to comment on the applicability of methods to dishes. Comments on the practicability of methods suggested are also desired.

## ACKNOWLEDGMENT.

The writer wishes to acknowledge the assistance of collaborators from the following laboratories: H. E. Weaver, Larabee Flour Mills; E. N. Frank, Washburn-Crosby Co.; D. A. Coleman, Bureau of Agricultural Economics; C. B. Morison, American Institute of Baking; and C. H. Bailey, University of Minnesota.

## COMMENTS ON METHODS USED.

The use of alundum (Method II) was suggested by Paul Smith and P. E. Minten of Indianapolis. The associate referee furnished collaborators with a sufficient quantity of R. R. alundum 60 mesh.

Method III, specifying calcium acetate was outlined by the writer (see 1924 report) and was also suggested by Weaver, St. Joseph, Mo.

The associate referee is indebted to Frank for suggestions used in outlining Method IV. He suggested the use of an acetic acid solution of calcium acetate and a stirring rod prepared by tightly rolling a piece of ashless filter. The acetic acid solution mixes readily with flour without formation of a dough, and the mixture can be ignited directly without much danger of frothing.

#### RESULTS OF COLLABORATIVE STUDIES.

The results obtained by the different collaborators for the four methods outlined are given in Tables 1-4. Table 5 compares results for the four methods, and Table 6 compares the time required for the different methods.

Results for Method II agree fairly well with results obtained by Method I. With one exception collaborators state that the use of alundum saved some time. The principal objection to the use of alundum in the writer's opinion is the danger of losing small particles, which would seriously affect the results. One collaborator objects to the use of alundum on the grounds that it is not possible to determine by appearance if the ashing is completed. The method is deemed worthy of further study.

The results obtained with calcium acetate methods were disappointing, but the collaborative studies brought out some important points regarding the use of calcium acetate as an aid to ashing.

Calcium acetate is added to flour to prevent fusion of the ash and thus permit the use of a higher temperature for ignition. The ash of both whole wheat and bolted flour contains an excess of acid forming elements. The principal acid forming radical in excess is probably phosphate from organic phosphates, and the excess phosphoric acid is probably present in ash as acid salts of potassium and sodium and in some cases as free phosphoric acid. The addition of calcium acetate in excess converts these salts into salts not easily fusible. The excess calcium acetate is presumed to be ignited to calcium oxide, but it is possible that when calcium acetate is ignited in the presence of carbon a part of the excess remains as calcium carbonate.

The variation in blanks and results indicates that calcium acetate is not completely reduced to calcium oxide.

For Method III, results obtained by collaborators 1, 2, and 6 checked fairly well with the official method. The low results for ash obtained by collaborators 3 and 4 were evidently due to the very high blank. The blanks obtained by collaborators 1, 5, and 6 agree very closely, and the low ash result for No. 5 is due to one very low duplicate (see Tables 3 and 4).

The associate referee is also indebted to Frank for additional data on the acetate methods. He finds that if the ash residues after removal

from the muffle furnace are heated in a gas flame an additional loss occurs, owing evidently, to the reduction of calcium carbonate to calcium oxide.

The effect of heating ash residues in a gas flame is shown by the following results obtained by Frank:

	AFTER HEATING IN MUFFLE		ADDITIONAL HEATING IN GAS FLAME	
	Weight of residue	Ash	Weight of residue	Ash
	<i>gram</i>	<i>per cent</i>	<i>gram</i>	<i>per cent</i>
Sample.....	0.0157	0.310	0.0138	0.450
Sample.....	0.0157	0.310	0.0138	0.450
Blank.....	0.0085		0.0048	
Blank.....	0.0085		0.0047	

The theoretical weight ratio of calcium oxide to calcium carbonate is 1 : 1.82, while the ratio of 0.0048 to 0.0085 is 1 : 1.77. The writer finds that the calcium acetate is completely reduced if exposed to a temperature in the muffle greater than 1000°C.

Frank also determined the effect of different ratios of acetate solution and flour. The data are given below.

RATIO	ASH AFTER HEATING IN MUFFLE AT 1050°F.	ASH AFTER ADDITIONAL HEATING OVER GAS FLAME
	<i>per cent</i>	<i>per cent</i>
3 cc. to 1 gram of flour.....	0.400	0.470
3 cc. to 2 grams of flour.....	0.360	0.450
2 cc. to 2 grams of flour.....	0.365	0.430

The greater the quantity of calcium acetate used the larger the percentage of ash was found to be. These data indicate that any large excess of calcium, over the quantity required to neutralize acid of ash, is difficult to reduce to calcium oxide.

The use of calcium acetate does permit the ashing to be made at high temperatures without danger of loss by volatilization, and by the use of a high temperature the ignition may be completed in a relatively short time. The addition of calcium acetate does, however, greatly increase the chance of error, since the acetate solution must be measured very accurately. The method suggested by Frank has a distinct advantage over previous acetate methods. The acetic acid solution of calcium acetate mixes very readily with the flour, and the mixture of flour and acetic acid can be heated without danger of frothing. The results of collaborative work on the acetate method for ash indicate, however, that further investigational work is necessary before such a method can be properly outlined. The collaborative work conducted this year has pointed out some of the difficulties to be encountered.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That collaborative study of the Bailey-Hertwig, or glycerol, method for ash be discontinued.

(2) That the referee make additional studies of the use of alundum for ashing flour, using different granulations.

(3) That the calcium acetate method suggested by Frank be subjected to further study.

TABLE 1.

*Results of ash determinations using Method I (Official Method).*

COLLABORATOR NUMBER	A	B	C	D	AVERAGE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.405	0.415			0.410
2	0.405	0.410			0.408
3	0.430	0.430			0.430
4	0.425	0.425	0.430	0.420	0.425
5a	0.390	0.390	0.400		0.393
5b	0.430	0.415	0.385	0.405	0.409
5c	0.460	0.420	0.450		0.440
6	0.420	0.430			0.425

TABLE 2.

*Results of ash determinations using Method II (alundum).*

COLLABORATOR NUMBER	A	B	C	D	AVERAGE
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.415	0.425			0.420
2	0.405	0.410			0.408
3	0.405	0.405			0.405
4	0.450	0.450	0.450	0.450	0.450
5a	0.390	0.390	0.385		0.390
5b	0.420	0.390			0.405
5c	0.370	0.420	0.410		0.400
6	0.400	0.390			0.395

TABLE 3.

*Results of ash determinations using Method III (water solution of calcium acetate).*

COLLABORATOR NUMBER	AVERAGE WEIGHT OF BLANK	ASH A	ASH B	AVERAGE
	<i>gram</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.0050	0.430	0.435	0.433
2	0.0071	0.415	0.425	0.420
3	0.0085	0.325	0.330	0.328
4	0.0084	0.300	0.305	0.303
5	0.0053	0.305	0.415	0.360
6	0.0051	0.430	0.410	0.420

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 89



TABLE 4.

*Results of ash determinations using Method IV.*

COLLABORATOR NUMBER	AVERAGE WEIGHT OF BLANKS	A	B	AVERAGE
	<i>gram</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	0.0055	0.425	0.425	0.425
2	0.0063	0.455	0.455	0.455
3	0.0085	0.360	0.360	0.360
4	0.0108	0.279	0.270	0.270
5	0.0050	0.400	0.310	0.360
6	0.0051	0.490	0.475	0.483

TABLE 5.

*Comparison of Methods I, II, III, and IV for ash determinations.*

COLLABORATOR NUMBER	PERCENTAGE OF ASH			
	Method I	Method II	Method III	Method IV
1	0.410	0.420	0.433	0.425
2	0.408	0.408	0.420	0.455
3	0.430	0.405	0.328	0.360
4	0.425	0.450	0.303	0.270
5a	0.393	0.390	0.360	0.360
5b	0.409	0.405		
5c	0.440	0.400	0.420	
6	0.425	0.393		0.483

TABLE 6.

*Total time required to obtain results on ash determinations using different methods.*

COLLABORATOR NUMBER	I TOTAL	II TOTAL	III TOTAL	IV TOTAL
	<i>hours</i>	<i>hours</i>	<i>hours</i>	<i>hours</i>
1	20½	5	3	1½
2	6½	5½	3	1½
3	16	3½	1½	1
4	67	19	18½	6
5	6½	6½	6½	6½
6	6	4½	2½	2½

No report on chlorine in bleached flour was given by the associate referee.

## REPORT ON GLUTENIN IN WHEAT FLOUR.

By M. J. BLISH<sup>1</sup> (Agricultural Experiment Station, Lincoln, Nebr.),  
*Associate Referee.*

Work on the determination of glutenin in wheat flour has been confined to making a critical study of the direct method proposed by Blish and Sandstedt<sup>2</sup>, which gives results similar to those obtained by the indirect method of Sharp and Gortner<sup>3</sup>. This work has consisted chiefly of noting results obtained by subjecting certain prescribed details of the method to variations and modifications, and these studies have led to some rather surprising results which point to the necessity for further work.

## EXPERIMENTAL.

The first point to be investigated was the effect of varying the weight of the flour sample used, the amounts and proportions of other reagents, as well as all manipulation, being the same as in the Blish-Sandstedt method. It was found that the apparent percentage of glutenin increased with the flour concentration as shown in Table 1.

TABLE 1.

*Effect of varying the flour concentration on percentage of glutenin estimated by the Blish-Sandstedt Method.*

WEIGHT OF FLOUR	NORMAL NaOH USED TO DISSOLVE FLOUR PROTEIN	0.2 N HCl REQUIRED TO PRECIPITATE GLUTENIN	GLUTENIN IN FLOUR
<i>grams</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
2	5.0	5.2	3.76
4	5.0	5.0	4.47
6	5.0		4.33
8	5.0	3.7	5.14
10	5.0	3.0	5.93
12	5.0	2.8	6.06

This experiment was repeated, 0.2 N phosphoric acid ( $H_3PO_4$ ) being used instead of 0.2 N hydrochloric acid to precipitate glutenin from the alkaline alcoholic protein solution. In a third similar experiment 5 cc. of normal potassium hydroxide instead of sodium hydroxide was used to extract the protein from the flour, while 0.2 N sulfuric acid was the neutralizing acid. The results are shown in Table 2.

Results presented in Tables 1-3 indicate that when the flour concentration is varied, while all other details of the method are carefully observed, the percentage of glutenin in the flour increases with the flour concentration, regardless of the nature of the alkali and acid employed.

<sup>1</sup> The analytical work herein reported was done by R. C. Abbott, Assistant Professor of Chemistry, University of Nebraska.

<sup>2</sup> *Cereal Chemistry*, 1925, 2: 57.

<sup>3</sup> *Minnesota Tech. Bull.* 19.

TABLE 2.

*Same as Table 1, except that  $H_3PO_4$  was used instead of  $HCl$  to precipitate the glutenin.*

WEIGHT OF FLOUR	NORMAL $NaOH$ USED TO DISSOLVE FLOUR PROTEIN	0.2 N $H_3PO_4$ USED TO PRECIPITATE GLUTENIN	GLUTENIN IN FLOUR
<i>grams</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
2	5	6.0	3.42
4	5	5.0	4.08
6	5	4.6	4.71
8	5	3.8	5.22
10	5	3.4	5.52
12	5	3.0	5.80

TABLE 3.

*Same as Table 1, except that  $KOH$  and  $H_2SO_4$  were used instead of  $NaOH$  and  $HCl$ .*

WEIGHT OF FLOUR	NORMAL $KOH$ USED TO DISSOLVE FLOUR PROTEIN	0.2 N $H_2SO_4$ USED TO PRECIPITATE GLUTENIN	GLUTENIN IN FLOUR
<i>grams</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
2	5	5.3	3.18
4	5	4.3	3.93
6	5	3.5	4.90
8	5	3.3	5.05
10	5	2.8	5.62
12	5	2.5	5.79

The next step was to run a series of determinations in which the same flour concentrations were used throughout, the only variation being in the concentration of the alkali used to extract the flour protein. The outcome of this experiment is shown in Table 4.

TABLE 4.

*Glutenin by Blish-Sandstedt method varied only by the concentration of alkali used for protein extraction.*

WEIGHT OF FLOUR	NORMAL $NaOH$ USED FOR EXTRACTION	0.2 N $HCl$ USED TO PRECIPITATE GLUTENIN	GLUTENIN IN FLOUR
<i>grams</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
8	2.5	1.6	5.87
8	5.0	3.4	5.04
8	7.5	5.4	4.10
8	10.0	7.1	3.76
8	12.5	9.1	3.76

The experiment reported in Table 4 indicates that as the alkali concentration is increased, all other details remaining constant, the percentage of glutenin recovered decreases.

The experiments reported in the first four tables indicate that the percentage of glutenin obtained will vary with the ratio of total protein to the concentration of hydrogen-ions during the protein extraction. In

order to substantiate this conclusion, a series of determinations was made in which both the flour and sodium hydroxide concentrations were varied, the ratio of flour to sodium hydroxide being in each case the same as in the published Blish-Sandstedt method. These results are shown in Table 5.

TABLE 5.

*Effect of varying both flour and NaOH concentrations, the ratios being the same as in the Blish-Sandstedt method.*

WEIGHT OF FLOUR	NORMAL NaOH USED FOR EXTRACTION	0.2 N HCl USED TO PRECIPITATE GLUTENIN	GLUTENIN IN FLOUR
<i>grams</i>	<i>cc.</i>	<i>cc.</i>	<i>per cent</i>
2	1.25	1.2	5.12
4	2.5	2.2	5.13
6	3.75	2.8	5.0
8	5.0	3.5	5.38
10	6.25	4.05	5.2
12	7.5	4.55	5.37

The data presented in Table 5 show that in the Blish-Sandstedt method both flour and alkali concentrations may be varied without affecting the results, providing the *ratio* of flour to alkali is kept constant. However, as Tables 1-4 show, an increase in the ratio of flour to alkali causes a decided increase in the percentage of glutenin obtained.

Since the percentage of glutenin was found to decrease as the ratio of flour to alkali decreased, and since an increasing quantity of alkali gives rise to an increasing quantity of sodium chloride when the glutenin is precipitated by adding hydrochloric acid, it was considered advisable to test the effect of salt by running a series by the prescribed method, but adding various quantities of sodium chloride just before precipitation of the glutenin. The results of this experiment are shown in Table 6.

TABLE 6.

*Effect of added salt (NaCl) on determination of glutenin by the Blish-Sandstedt method.*

NaCl ADDED TO PROTEIN EXTRACT	GLUTENIN IN FLOUR
<i>grams</i>	<i>per cent</i>
2.0	3.11
1.5	3.16
1.0	3.45
0.5	3.90
0.25	4.39
0.12	4.53
0.08	4.70
0.04	4.93
None	5.16

Table 6 shows that the quantity of salt present when the glutenin is precipitated can exert a very noticeable influence on the quantity of

glutenin obtained. The effect of varying ratios of flour to alkali, as presented in Tables 1-5 cannot, however, be ascribed to differences in salt concentration, for when these differences were calculated from the quantities of hydrochloric acid used to precipitate the glutenin, it was found that the slight salt variation could account for but small fractions of the glutenin variations caused by changing the ratio of flour to alkali.

The next step was to determine whether or not slight variations in the final pH would exert any appreciable influence on the completeness of precipitation of glutenin, following the prescribed method in all other details. The results of this experiment are set forth in Table 7.

TABLE 7.

*Effect of varying pH of precipitation on percentage of glutenin estimated by the Blish-Sandslett method.*

0.2 N ACID USED FOR PRECIPITATION	ESTIMATED pH (BROM THYMOL BLUE)	RATE OF SETTLING	GLUTENIN IN FLOUR
cc.			per cent
3.8	6.6-6.8	4	5.16
4.0	6.4-6.6	1	5.13
4.2	6.2-6.4	3	5.18
4.5	6.0-6.2	2	5.18

The results in Table 7 appear to show that great care in adjusting the pH of the glutenin precipitation is not necessary, the only effect being possible slight differences in the rate of settling of the precipitated glutenin.

During the course of the experiments herewith reported it was found that glutenin may be very satisfactorily precipitated from alkaline alcoholic extracts of flour by passing carbon dioxide through the filtered extract. This converts the excess sodium hydroxide rapidly to sodium carbonate, and at the pH which results (about 6.6, as indicated by brom thymol blue), the glutenin is sharply precipitated, and settles rapidly, leaving a clear supernatant liquid. Whether or not some albumin or globulin, or both, are also precipitated by this procedure remains to be ascertained. The procedure gives results similar to those obtained by neutralizing the alkaline alcoholic extracts with hydrochloric acid, the results usually being slightly higher if carbon dioxide is used. Table 8 shows results of comparative tests by the two methods when used on the same flour. The work reported in the table is also calculated to indicate any possible effects from allowing the flour to stand for varying periods of time in contact with the water and alkali during the preliminary stage of the extraction, before the alcohol is added. In other respects the prescribed method was followed.

The average of the above figures shows slightly higher results where carbon dioxide was the precipitating agent. It furthermore appears that

TABLE 8.

*Comparison of CO<sub>2</sub> with HCl for precipitating glutenin from alcoholic alkaline extracts of flour protein. Also effect of time of standing in water and NaOH on glutenin results.*

TIME OF STANDING WITH WATER AND NaOH	GLUTENIN (NEUTRALIZING WITH HCl)	GLUTENIN (NEUTRALIZING WITH CO <sub>2</sub> )
<i>hours</i>	<i>per cent</i>	<i>per cent</i>
1*	5.44	5.61
1	5.25	5.39
2	5.33	5.73
4	5.13	5.67
6	5.22	5.50
8	4.96 (?)	5.73
24	5.42	5.28
Average	5.25	5.56

\* 15 minutes was found to give complete extraction of flour protein.

varying the length of time which the flour stands in contact with water and sodium hydroxide, during the first stage of the extraction, has little effect on the final results, up to 24 hours.

An experiment was then made to ascertain whether or not varying the ratio of flour to sodium hydroxide would affect the glutenin results when carbon dioxide was used as a precipitant to the same extent as was the case when the glutenin was precipitated by hydrochloric acid (see tables 1-5). Table 9 presents the results of this experiment.

TABLE 9.

*Effect of varying ratio of flour to NaOH, and precipitating glutenin with CO<sub>2</sub>.*

WEIGHT OF FLOUR	NORMAL NaOH USED FOR PROTEIN EXTRACTION	GLUTENIN IN FLOUR
<i>grams</i>	<i>cc</i>	<i>per cent</i>
8	2.5	5.81
8	5.0	5.44
8	7.5	5.24
8	10.0	4.93

When the results presented in Table 9 are compared with those in Table 4, it may be noted that the variation in percentage of glutenin resulting from changing the flour to sodium hydroxide ratio is decidedly less where carbon dioxide is the precipitating reagent than is the case where the glutenin is precipitated by the neutralization with strong acid.

#### DISCUSSION.

It would appear from the work here reported that the absolute quantitative accuracy of the glutenin method of Blish and Sandstedt is not yet definitely and finally established. It gives results which are closely comparable with results of the indirect method of Sharp and Gortner.

Its approximate accuracy is also indicated by the ammonia fractions obtained from the hydrolysis of both the precipitated and non-precipitated protein fractions resulting from the prescribed procedure. However, the ammonia fraction of the hydrolyzed protein cannot yet be accepted as an *absolute* criterion, since the percentage of ammonia nitrogen that *perfectly pure* glutenin should contain is not definitely established, nor is it absolutely certain that all glutenins contain exactly the same percentages of ammonia nitrogen. Cross and Swain<sup>1</sup> report ammonia nitrogen in a number of glutenin preparations, and these figures range from 12.88 to 16.15 per cent, the majority, however, being between 15 and 16 per cent. Blish<sup>2</sup> reported 16.50 and 16.17, respectively, on two glutenin preparations. Osborne<sup>3</sup>, however, reports 18.8 per cent ammonia nitrogen in glutenin. Blish and Sandstedt found 15.91 per cent in the protein precipitated by their method, and this figure agrees closely with the average of the majority of published analyses. The same condition exists, although to a lesser degree, with gliadin. Published analyses of gliadin indicate that its content of ammonia nitrogen may be anywhere from about 24 to 26 per cent, the average probably being around 25 per cent. Blish and Sandstedt found 23.9 per cent of ammonia nitrogen in their non-precipitated fraction, and state that "such a value could be reasonably expected from the hydrolysis of gliadin contaminated by the small amounts of albumin and globulin that are always present in wheat flour". It is upon these data, and the fact that the results of the Blish-Sandstedt method agree with those obtained by Sharp and Gortner's method, that the accuracy of the Blish-Sandstedt method depends at the present time.

Because variations in the ratio of flour to alkali concentration, following Blish and Sandstedt's procedure, so decidedly affect the glutenin results, it must be considered extremely fortunate if their prescribed ratio of flour to alkali concentration happens to be the correct one, even though its *approximate* accuracy is indicated by the facts stated in the preceding paragraph. There are several doubtful points which should be settled, among which are the following: Why do variations in the ratio of flour to alkali concentration cause such certain regular variations in glutenin results? Why do the lower ratios of flour to alkali concentration cause correspondingly lower glutenin results? Which ratio of flour to alkali gives the most nearly correct glutenin results? Do the "glutenin precipitates" have the same chemical composition regardless of the ratio of flour to alkali used in their preparation? If so, in the case of the lower yields, what happens to the glutenin which is rendered non-precipitable by the higher alkali concentrations? Is the carbon dioxide precipitation method better than the one with strong acid, and why?

<sup>1</sup> *Ind. Eng. Chem.*, 1924, 16: 49.

<sup>2</sup> *Ibid.*, 1916, 8: 138.

<sup>3</sup> *The Vegetable Proteins*. 1924. Longmans, Green and Co., New York.

What happens to the albumin and globulin under the various treatments? Alcoholic alkaline extracts of flour always give off hydrogen sulfide when these extracts are neutralized with either strong acid or carbon dioxide. Where does this come from, and what are the consequences of this "action of alkali on the sulfur-containing portion of the flour protein"?

These and other questions must be answered before an absolute method involving the use of alkaline reagents can be positively assured. Some information bearing on several of these points has been obtained in this laboratory, but much more work is necessary before definite statements can be made. Further work is in progress.

#### RECOMMENDATION<sup>1</sup>.

It is recommended that the work on the determination of glutenin in wheat flour be continued along the lines indicated by the results of the work herewith reported, with a view toward clearing up some of the doubtful points indicated in the preceding paragraph.

#### REPORT ON METHODS FOR SAMPLING FLOUR.

By H. RUNKEL (U. S. Food and Drug Inspection Station, Minneapolis, Minn.), *Associate Referee*.

The report<sup>2</sup> of the Committee on Sampling, which was approved at the last meeting of the association, contained six general and fundamental considerations to be followed in any experimental studies which might be undertaken on the subject of sampling. The committee also recommended that whenever possible, cooperative arrangements be made by the associate referees under the subject of sampling with the various industries involved, as well as with any commercial group of chemists connected with such industries, looking toward their active cooperation in the development of the most desirable methods of sampling.

The efforts of the associate referee were directed toward the formulation of a proposed tentative method for sampling flour, in which the fundamental considerations presented by the committee, as well as the pertinent facts presented by other investigators, are properly applied to maintain a reasonable balance between theory and practice.

In order to facilitate consultations and cooperation with the associate referee the officers of three organizations very kindly designated the following representatives:

M. A. Gray, Chief Chemist, Pillsbury Flour Mills Co., Minneapolis, Minn., representing the Millers' National Federation.

Leslie R. Olsen, Chief Chemist, International Milling Co., Minneapolis, Minn., and

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 90.

<sup>2</sup> *This Journal*, 1925, 8: 287.



D. A. Coleman, U. S. Bureau of Agricultural Economics, Washington, D. C., both representing the American Association of Cereal Chemists.

C. B. Morison, Assistant Director, American Institute of Baking, Chicago, Ill.

Through the cooperation of these representatives the viewpoints of the various organizations were obtained. Ways for securing further information were agreed upon. The various types of triers and containers and other similar essential details were considered. The formulation of the proposed tentative method was given careful attention by each representative, and many helpful comments were received. Through them and by direct correspondence the officials of the three organizations have given whole hearted cooperation, which was of inestimable value in maintaining a proper balance between theory and practice.

In addition to the representatives named, and at their suggestions, ten millers, three bakeries, ten commercial exchanges, and three commercial chemists were consulted by correspondence. Their assistance was also highly appreciated and very valuable. They submitted descriptions of methods of sampling in use by the trade at the present time. Grateful acknowledgment is also made to Raymond Hertwig for his helpful suggestions for the method of investigation and to the numerous other individuals not mentioned in this report who contributed suggestions and ideas by conference and correspondence.

#### VARIATION IN COMMERCIAL METHODS.

As the various methods in use by the trade were outlined in correspondence and verbally, it became apparent that the considerable variation in commercial methods of sampling was according to the purpose for which the sample was to be used. While the methods described in most cases had apparently been worked up to suit a particular purpose or the particular needs of an organization, it was evident at once that the variation occurred principally in detail and not in principle. For example, one baking chemist stated that he designates the number of sacks to be sampled from a given number in a lot, that he uses a flour thief for taking samples, that the thief is inserted in the sacks diagonally from the top of the sack, and that he uses a tin can as a container. One miller stated that he takes a sample from each twenty-five barrels of flour, one sack from the bottom, one from the top, and one from the middle of the pile, inserting a grain probe diagonally through each sack. Numerous other methods were described, but it was noted that more or less consideration was given in each instance to the purpose of sampling, to the number of samples to be drawn, the location of the sacks to be sampled, the method of drawing the sample from the sack and the method of preserving the sample after it was drawn. These points corresponding very closely to the six general considerations of the Committee on Sampling, they were accordingly made the subject of study. The method as submitted covers each one.

## FACTS RELATING TO PROPOSED METHOD.

Mention of some pertinent facts may suggest reasons for some of the principles and details of the proposed method.

The square root of the number of the sacks in the pile mentioned in the first paragraph of the method has no theoretical significance. It represents about a mean of the opinions of investigators and samplers. The statement is easily remembered.

Roethe<sup>1</sup> has shown that about half the flour in a 140 pound sack lies in the outer zone, 1½ inches wide. Morton<sup>2</sup> has shown, and Paul<sup>3</sup> has confirmed the fact, that after several days' dry storage the flour in the inner zone of a sack contains more moisture than that in the outer zone. Theory would require that the sample consist of a proportion of each zone equivalent to their relative volumes. The nearest balance between theory and practice which could be discovered is incorporated in the method that specifies that a core be drawn from the top corner to the center and another from the other corner half the distance to the center of the sack.

Numerous preliminary tests were made of various triers. Difficulty was found in removing a complete core. The most adaptable trier appeared to be the one mentioned in the method, which is further described by Roethe as the 30 inch tubular trier. Very simple instructions can be given for its use. As far as could be ascertained it is specially made by a firm in Boston at a cost of \$15.00. It is used to some extent on commercial exchanges<sup>4</sup> in the eastern part of the United States.

Paul has shown that after a few days' dry storage the sacks in the outer part of a pile of flour contain less moisture than the inner sacks. The sampling in the proportion of 4 : 3 : 2 : 1 sacks from the most to the least exposed portions of the pile agrees for practical purposes with theoretical requirements. The ratio is easily remembered. The sum of the numbers is ten, which makes a simple base for computation.

The suggestion has been made that all these samples be combined into one composite. In some determinations this is necessary in order to secure a sufficient quantity for the test. This alternative was provided for in the last paragraph of the method. However, analysis of separate subdivisions permits comparison of the moisture content of the individual sack with the weight so that the amount of shrinkage may be properly estimated. This procedure is highly desirable in official and referee work, particularly in the case of disputes. The average of the analytical results on all the samples taken from one pile, according to the method, would give the average composition of the entire pile.

<sup>1</sup> *This Journal*, 1925, 8: 424.

<sup>2</sup> *Ibid.*, 680.

<sup>3</sup> Unpublished.

<sup>4</sup> Personal correspondence, 1925.

Bailey<sup>1</sup> has shown that flour in equilibrium with air at 25°C. and 70 per cent humidity contained 12.05 per cent moisture, but that it contained 7.92 per cent moisture when subjected to the same temperature at 50 per cent humidity. Morton found that flour should be mixed at approximately 55 per cent relative humidity as at higher or lower humidities marked gains or losses in weight are likely to take place. No data have been found to indicate the speed with which flour takes up or loses moisture, but the facts submitted show the necessity for transferring the sample to an air-tight container and drawing samples from an undisturbed portion of the sack and pile without unnecessary delay.

The method as formulated by the associate referee has been published<sup>2</sup>.

The original plan of study proposed that the method in its present form be submitted to the members of the cooperating organizations for suggestions and criticisms. While the method has been passed upon by the officers and in one case by members of the Executive Committee, the associate referee was unable, because of lack of time, to secure the valuable assistance which might have been obtained from the various members of these organizations. The officers have indicated, however, that this cooperation may be obtained later.

The method should be tried out and reported upon by a number of samplers. Some collaboration should later be secured to furnish an adequate basis for a judgment as to the proper valuation of the method finally adopted.

#### RECOMMENDATION<sup>3</sup>.

It is recommended that the method submitted be adopted as a tentative method for sampling flour.

#### REPORT ON METHODS FOR THE EXAMINATION OF BREAD.

By L. H. BAILEY (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

The associate referee did not attempt to cover the whole subject of the study of bread analysis this year, but selected methods for sampling, total solids, lipoids, and lipid phosphoric acid for first consideration.

The most logical method of obtaining for analysis a representative sample of a loaf of bread seemed to be to air-dry the entire loaf, grind, and mix. Such a procedure was followed. No subdivision method would, under all circumstances, yield correct proportions of crumb and crust and, at the same time, have a moisture content that was representative of the entire loaf. Hence, other methods of sampling were not tried.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1920, 12: 1102.

<sup>2</sup> *This Journal*, 1926, 9: 39.

<sup>3</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 89.

In making the determinations of total solids, consideration was given to the fineness of the particles in connection with drying at different temperatures and pressures. As a referee method of drying, selection was made of the proposed "umpire" method<sup>1</sup> which was considered by this association last year for determining moisture in flour. This method stipulates that the samples be dried at a temperature of 98°-100°C. in a partial vacuum having a pressure of not more than 25 mm. of mercury for approximately 5 hours or until the samples cease to lose weight.

An attempt was made to duplicate the results obtained by the umpire method by drying the samples at atmospheric pressure and at somewhat higher temperatures and for different periods of time. Samples were first dried for 1 hour at 130°C., but as there was such a marked color change in them when subjected to this temperature, it was decided to use a lower temperature and longer time. Next, a temperature of 115°C. was used, and the time was lengthened to 2, 3, and 4 hours. With three different loaves of bread, the samples dried at 115°C. for 3 hours yielded results which checked closely with those obtained by drying at a pressure of 15-20 mm. for 5 hours and at a temperature of 98°-100°C. Samples ground fine enough to pass a 20-mesh sieve, and the same material ground fine enough to pass a 40-mesh sieve, were dried by the methods described. The results of this study were published<sup>2</sup>.

#### COLLABORATIVE WORK.

After doing this preliminary work, request was made for collaborative data on these methods as well as for data on lipoids and lipid phosphoric acid by the two methods previously described<sup>3</sup>. These two methods have been adopted by this association for use in different types of food products, and it was desired to ascertain which is the more suitable for bread analysis. Request for collaborative study was made of six of the branch stations of the Bureau of Chemistry and of the American Institute of Baking. Only three of the branch stations submitted results, and two of these did not determine the lipid phosphoric acid.

Each laboratory was asked to supply itself with a loaf of fresh bread and make the preliminary air-drying, as well as the final drying of the sample. Because these different samples were used, comparisons cannot be made between the results obtained by the different collaborators.

From the few results obtained, no conclusions can be drawn as to which methods are preferable for the determinations of lipoids and lipid phosphoric acid.

As to the determination of total solids, with one exception the collaborators obtained slightly higher results by drying their samples at

<sup>1</sup> *This Journal*, 1925, 8: 665.

<sup>2</sup> *Ibid.*, 685.

<sup>3</sup> *Ibid.*, 1924, 8: 109, 116.

atmospheric pressure and at a temperature of 112°–117°C. for 3 hours than they did by drying them for 5 hours at 98°–100°C. at greatly reduced pressure. The referee also dried his sample at 130°C. for 1 hour at atmospheric pressure and secured results which agreed very closely with those obtained by drying for 5 hours at 98°–100°C. and at 5 mm. pressure. However, the sample dried at atmospheric pressure had changed color very decidedly, although there was no evident material change in weight accompanying this change in color.

TABLE 1.  
*Bread analysis.*

ANALYST	TOTAL SOLIDS		LIPIDS		LIPOID P <sub>2</sub> O <sub>5</sub>	
	Drying 5 hours at 98°–100°C. 25 mm. or less Hg pressure	Drying 3 hours at 112°–117°C. atmospheric pressure	p. 109*	p. 116	p. 109	p. 116
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Carlos A. Greenleaf	64.02	64.30	2.38	4.09	0.023	0.037
Food and Drug Inspec- tion Station, Cincinnati, Ohio.	64.01	64.25	2.37	4.07	0.023	0.036
	(30 mm. Hg)					
John T. Field	64.16	64.05	3.03	2.55	....	....
Food and Drug Inspec- tion Station, Minneap- olis, Minn.	64.12	64.02	3.00	2.49		
	(50 mm. Hg)					
J. C. Palmer	66.68	66.90	6.38	7.11	....	....
Food and Drug Inspec- tion Station, San Fran- cisco, Calif.	66.70	66.74	6.32	7.16		
P. L. Gerber      Sample	65.12	65.33	....	....	....	....
American      1 A	65.18	65.43				
Institute of      Sample	65.10	65.30	....	....	....	....
Baking      1 B	64.96	65.32				
Chicago, Ill.						
W. C. Luckow      Sample	65.31	65.71	....	....	....	....
American      1 A	65.30	65.72				
Institute of      Sample	65.32	65.62	....	....	....	....
Baking      1 B	65.20	65.60				
Chicago, Ill.	65.28	65.62				
	65.24	65.61				
	(5 mm. Hg)					
L. H. Bailey	64.78	65.06	4.84	4.72	0.040	0.037
Bureau of Chemistry	64.86	65.06	4.79	4.70	0.040	0.037
Washington, D. C.		64.88/1 hr. at 64.88/130°C.				

\* Page numbers refer to Vol. 8, No. 2 of *This Journal*.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

- (1) That further study be made of a routine method for determining total solids in bread.
- (2) That methods for preparation of sample and determination of total solids by the proposed "Umpire" vacuum method be made official.
- (3) That the methods for determining lipoids and lipid phosphoric acid again be submitted to comparative collaborative study.

REPORT ON FAT (BY ACID HYDROLYSIS), LIPOIDS AND  
LIPOID-PHOSPHORIC ACID ( $P_2O_5$ ), AND WATER-SOLU-  
BLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER  
CENT ALCOHOL, IN FLOUR.

By SAMUEL ALFEND<sup>2</sup> (U. S. Food and Drug Inspection Station, St. Louis, Mo.), *Associate Referee*.

Of the four methods recommended for study by the associate referee this year, three were subjected to collaborative study. The fourth method, for the determination of unsaponifiable matter, was worked out by the Food Control Laboratory, Bureau of Chemistry, too late in the year to be studied collaboratively.

The methods submitted to the collaborators are those tentative for alimentary pastes<sup>3</sup>.

A batch of patent flour was carefully mixed, and sub-samples were placed in quart Mason jars and sent to the collaborators.

The associate referee wishes to express his appreciation of the careful work performed by the following analysts, who cooperated in these studies:

- L. H. Bailey, Food Control Laboratory, Bureau of Chemistry, Washington, D. C.
- J. H. Bornmann, U. S. Food and Drug Inspection Station, Chicago, Ill.
- R. T. Elliot, U. S. Food and Drug Inspection Station, Seattle, Wash.
- Raymond Hertwig, Food Control Laboratory, Washington, D. C.
- L. C. Mitchell, U. S. Food and Drug Inspection Station, St. Louis, Mo.
- J. C. Palmer, U. S. Food and Drug Inspection Station, San Francisco, Calif.
- G. C. Spencer, Bureau of Chemistry, Washington, D. C.

The results obtained are given in Table 1.

## DISCUSSION OF RESULTS.

*Fat Method.*

The general agreement is quite satisfactory. It is of interest to note that both of the analysts whose results were somewhat high reported

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 91

<sup>2</sup> Presented by L. C. Mitchell.

<sup>3</sup> *Methods of Analysis*, A. O. A. C. 1925, 232-7

TABLE 1.  
Results obtained by collaborators.

ANALYST	FAT BY ACID HYDROLYSIS	LIPIDS	LIPID $P_2O_5$	WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	
L. H. Bailey	1.36 1.30 1.38	1.20 1.20	0.033 0.034	0.034 0.034 0.034 0.042
J. H. Bornmann	1.40 1.38	1.20 1.20	0.036 0.037	0.018 0.021
R. T. Elliot	1.57 1.53	1.25 1.25	0.034 0.035	0.017 0.018
R. Hertwig	...	1.20 1.24 1.25	0.032 0.032 0.033	0.027 0.028 0.034
L. C. Mitchell	1.38 1.35 1.40	1.37 1.40 1.44 1.46	0.044 0.044 0.044 0.046	0.036 0.034
J. C. Palmer	1.60 1.60	1.15 1.17	0.035 0.036	0.028 0.040
G. C. Spencer	1.52 1.27	1.31 1.25	0.041 0.043	0.028 0.028
S. Alfend	1.37 1.38 1.40	1.28 1.28 1.30 1.31	0.038 0.038 0.037 0.040	0.032 0.034 0.038 (0.19 0.22 0.34)*

\* Obtained with the use of paper pulp on a small Büchner funnel. The other three results were obtained by the method given in this report.

that their blanks were zero, whereas the blank obtained by several other analysts was about 0.1–0.2 per cent. The results of these analysts were also 0.1–0.2 per cent higher.

#### *Lipids and Lipoid Phosphoric Acid Methods.*

With one exception the results submitted agree quite closely. Mitchell, whose results are slightly high, suggests that the discrepancy might be due (1) to digesting the sample in alcohol for over an hour, instead of for 15 minutes, as directed; (2) failure to filter free of all impurities after dissolving the crude residue in chloroform; (3) failure to dry the lipoids to minimum weight. He observed that after 75–90 minutes, continued heating of the lipoids resulted in a steady increase in weight, amounting to 0.03–0.04 per cent an hour.

The associate referee agrees with the comments of several collaborators who point out that the differences in the results obtained on lipid

phosphoric acid ( $P_2O_5$ ), are little greater than might be expected when different analysts are working on the same samples of an inorganic phosphate.

*Water-Soluble Protein-Nitrogen Precipitable by 40 Per Cent  
Alcohol Method.*

Although the relative differences in the results on this method appear great, in actual percentage of the sample they are quite small. The associate referee feels that these errors may be obviated by eliminating the use of paper pulp and using a thin asbestos pad on a Hirsch funnel, or where possible, on a Gooch crucible. In this way the large and variable blank may be done away with.

METHODS.

In the light of the experiences encountered during the year's studies, several slight changes in the methods are suggested in the interest of greater clearness and perhaps greater accuracy. No change of principle is involved. The methods as submitted now are as follows:

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL.

REAGENTS.

(a) *40 per cent alcohol*.—Mix 50 volumes of water and 35 volumes of 95 per cent alcohol.

(b) *Asbestos*.—Ignite and rub through an 8-mesh sieve.

DETERMINATION.

Place 20 grams of the flour in a 200 cc. nursing bottle, add 100 cc. of water from a pipet, shake the bottle violently a few times to prevent lumping of the sample, and add exactly 100 cc. more of water. Shake the stoppered bottle in a mechanical shaker (if not available, shake by hand) for 30 minutes. The temperature of the water should not exceed  $30^{\circ}C$ . Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Replace the asbestos if it clogs. The filtrate should be practically clear. Pipet 100 cc. of the filtrate into a 250 cc. beaker-flask or Erlenmeyer flask. Add 1.2 grams of sodium chloride and dissolve. Add 0.1 gram of asbestos, shake, and with constant agitation add 70 cc. of 95 per cent alcohol. Allow to stand overnight. Filter the mixture through a thin pad of asbestos in a Gooch crucible, using light suction. Wash the flask and precipitate twice with the 40 per cent alcohol. Transfer the filter mat with the precipitate to a Kjeldahl flask, and determine the nitrogen as directed on p. 8, 22<sup>1</sup>, collecting the ammonia in 10 cc. of 0.1 *N* acid. Make blank determinations on the reagents, using as nearly as possible the same quantity of asbestos.

FAT (ACID HYDROLYSIS METHOD).

Place 2 grams of the flour in a 50 cc. beaker, add 2 cc. of 95 per cent alcohol, and stir so as to moisten all particles. (The moistening of the sample with alcohol prevents lumping on addition of the acid.) Add 10 cc. of dilute hydrochloric acid (25 + 11), mix well, set the beaker in a water bath held at  $70^{\circ}$ – $80^{\circ}C$ ., and stir at frequent intervals

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925



for 30-40 minutes. Add 10 cc. of 95 per cent alcohol and cool. Transfer the mixture to a Röhrig or Mojonnier fat extraction apparatus. Rinse the beaker into the extraction tube with 25 cc. of ether previously washed with water, in three portions, and shake the mixture well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.) and mix well. Let stand until the upper liquid is practically clear. Draw off as much as possible of the ether-fat solution through a filter consisting of a pledget of cotton packed just firmly enough in the stem of a funnel to allow free passage of the ether into a weighed 125 cc. beaker-flask containing some porcelain chips or broken glass. Before weighing the beaker-flask dry it in an oven at the temperature of boiling water and then allow it to stand in the air until constant weight is attained. Re-extract the liquid remaining in the tube twice, each time with only 15 cc. of each ether. Shake well on the addition of each ether. Draw off the clear ether solutions through the filter into the same flask as before and wash the tip of the spigot, the funnel, and end of the funnel stem with a few cc. of a mixture of two ethers in equal volumes free from suspended water. Evaporate the ethers slowly on a steam bath, then dry the fat in a boiling water oven until it ceases to lose weight (approximately 75 minutes). Remove the flask from the oven, allow it to stand in the air until no further change in weight takes place, and weigh. Correct this weight by a blank determination on the reagents used.

#### LIPOIDS AND LIPOID PHOSPHORIC ACID.

Add 15 cc. of alcohol, 70 per cent by volume, to 5 grams of the flour, in a 200 cc. nursing bottle. Give the bottle a gentle rotary motion so as to moisten all the particles with the alcohol and set in a water bath kept at 75°-80°C. Heat for 15 minutes with frequent mixing by the same rotary motion. Add 27 cc. of 95 per cent alcohol, stopper the bottle, and shake vigorously for 2 minutes. Cool, add 45 cc. of ether, and shake well for 5 minutes. The sample should now be in a fine state of division. Centrifugalize just sufficiently to throw the solid particles out of suspension but not so as to pack the sample too firmly. Decant the liquid into a 250 cc. beaker containing some bits of broken porcelain or glass, and rinse off the bottle neck with ether. Re-extract the sample with three successive 20 cc. portions of ether, shake 1 or 2 minutes each time, centrifugalize, and decant into the beaker containing the first extract. Evaporate the combined ether-alcohol extracts just to dryness on the steam bath. Drive off any remaining moisture on the sides of the beaker by placing in a boiling water oven for 5 minutes. Dissolve the dry extract in approximately 15 cc. of chloroform and filter the solution into a previously dried and weighed platinum dish through a pledget of cotton packed in the stem of a funnel. Free with a glass rod any solid extract adhering to the beaker and transfer through the filter into the first washings by means of chloroform from a wash bottle all extract from the beaker bottom and sides. Finally wash the funnel and stem tip. The filtrate should be perfectly clear. Evaporate the chloroform on a steam bath and dry the dish and contents in a boiling water oven until no more weight is lost (75-90 minutes). Weigh. Report the extract as lipoids.

Dissolve the lipoids in 5-10 cc. of chloroform, add 5-10 cc. of 4 per cent alcoholic potassium hydroxide solution, evaporate to dryness on a steam bath, and char well in a furnace at a faint red heat. Cover the dish with a cover glass, add sufficient dilute nitric acid (1 + 9) to make the solution slightly acid, warm on a steam bath, and filter. Determine phosphoric acid in the filtrate as directed on p. 3, 7 or 10<sup>1</sup>. Report as lipid phosphoric acid (P<sub>2</sub>O<sub>5</sub>).

#### RECOMMENDATIONS<sup>2</sup>.

In view of the results obtained in this year's collaborative study and

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925.

<sup>2</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 89.

of the fact that these methods have been satisfactory as tentative methods for alimentary pastes, it is recommended—

(1) That the method for water-soluble protein-nitrogen precipitable by 40 per cent alcohol, as described in this report, be adopted as an official method for flour.

(2) That the method for fat (by acid hydrolysis), as described in this report, be adopted as an official method for flour.

(3) That the method for lipoids and lipid phosphoric acid, as described in this report, be adopted as an official method for flour.

(4) That the modified Kerr-Sorber method for unsaponifiable matter, as worked out in the Food Control Laboratory, Bureau of Chemistry, be subjected to collaborative study.

## REPORT ON HYDROGEN-ION CONCENTRATION OF FLOUR.

By C. H. BAILEY<sup>1</sup> (Minnesota Agricultural Experiment Station, St. Paul, Minn.), *Associate Referee*.

The appointment of an associate referee to study the determination of hydrogen-ion concentration of flour marks a new departure in the work of the association. No program of study of this determination has been carried along from previous years, and the associate referee has accordingly been obliged to block out the preliminary work in this field.

Several years ago the associate referee, with the collaboration of Anna C. Peterson<sup>2</sup>, studied the hydrogen-ion concentration of extracts of flours prepared in various ways. As a result of these studies it was concluded that varying the time of extraction from one to six hours and varying the temperature of extraction from 0°C. to 60°C. did not result in any substantial modification of the observed hydrogen-ion concentration of the extracts. Subsequent studies further indicated that the ratio of flour to water used by the collaborators in preparing these extracts likewise had a small influence upon the observed pH of the extract. When the buffer action of the extract was to be determined, it was found that the conditions of extraction must be very carefully controlled, however, since any substantial deviation from the procedure which was arbitrarily decided upon resulted in a modification of the buffer action.

With these earlier observations in mind, it seemed probable that the variations in practice that were likely to be encountered in different laboratories would not occasion any substantial variation in the findings. It was accordingly decided to distribute a number of flour samples to a dozen or more collaborators and to request these collaborators to proceed to determine the hydrogen-ion concentration of the material after

<sup>1</sup> Presented by M. J. Blah.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1921, 13: 916-918.

the method in vogue in their particular laboratories. The collaborators were also requested to furnish all details concerning the method which they employed.

#### DESCRIPTION OF SAMPLES.

The three samples of flour selected for these collaborative studies may be described as follows: (1) straight grade flour (unbleached), milled from hard spring wheat in the Minnesota State Experimental Flour Mill; (2) same flour as No. 1 after treatment with chlorine at the rate of one ounce per barrel of 196 pounds; (3) a 25 per cent extraction clear grade flour (unbleached), milled from hard spring wheat at the Minnesota State Experimental Flour Mill. The samples were distributed in small rubber bags or toy balloons, which constituted convenient mailing receptacles. The balloons held 55 grams of flour, when sealed were practically moisture-tight, and were of course much less fragile than the conventional glass bottle. So far as appears from the correspondence all the rubber bags reached the collaborators in good order. From this experience it appears that such rubber bags or pouches can be conveniently used in the distribution of samples of other like material for collaborative study, particularly when the hygroscopicity of the material is a factor to be considered.

Eleven collaborators reported the results of the determination of the hydrogen-ion concentration of these three samples of flour. Their findings, together with a brief summary of the procedure followed, will be found in Table 1. It appears that the ratio of flour to water used in preparing extracts varied widely, ranging from 10 grams of flour per 100 cc. of water to 20 grams per 100 cc. of water. The majority of the collaborators used 10 grams of flour per 100 cc. of water. There was likewise considerable variation in the time of extraction. Of the nine collaborators who reported on the extraction period, four extracted the flour for 30 minutes and three extracted for 60 minutes. One collaborator agitated the mixture of flour and water for ten minutes and then precipitated the suspended water particles in a centrifuge.

It was difficult to present in tabular form the treatment to which the mixture of flour and water was subjected before the extract was introduced into the hydrogen electrode vessel. In certain instances the extract was whirled in the centrifuge to precipitate the flour particles, and the supernatant extract was then filtered. In other instances the flour particles were allowed to settle out of suspension, and the extract was merely decanted into the electrode vessel. Two of the collaborators reported results obtained by introducing the suspension into the electrode vessel. Thus Collaborator No. 8 observed a higher hydrogen-ion concentration in the suspension than in the extract freed from the suspended flour particles.

In every instance but one, the mixture of flour and water was allowed to stand at about room temperature. In certain instances the water was brought to 25°C. before mixing with the flour, and the mixture was held at that temperature during the extraction period. Collaborator No. 4 held the mixture at a temperature of about 0°C. by immersing the flask containing the mixture in ice water.

Five of the nine collaborators using the conventional hydrogen electrode vessel reported that saturated potassium chloride was used in the calomel half-cell, while four of the collaborators used normal potassium chloride solution. Six of these same nine collaborators employed the Bailey hydrogen electrode vessel, while two collaborators used the Hildebrand type. The type of electrode used by the eighth collaborator was not indicated.

#### DISCUSSION OF RESULTS.

Two collaborators reported results obtained through the use of the quinhydrone electrode. It will be noted that in both of these instances the findings were substantially lower in terms of pH than were reported by the other nine collaborators who used the conventional hydrogen electrode vessel.

Fair agreement is to be observed in the instance of the collaborative reports of the first nine collaborators. In the instance of flour No. 1 these results range between  $\text{pH} = 6.11$  and  $\text{pH} = 6.26$ . Excluding one determination, the results agreed within 0.1 unit in terms of pH. In the instance of flour No. 2 the agreement was not so good, a total range of 0.27 units in terms of pH being recorded. Of the first nine reports, six range between  $\text{pH} = 5.92$  and  $\text{pH} = 5.99$ , which may be regarded as a satisfactory agreement. The reports on the third or clear flour varied from  $\text{pH} = 6.07$  to  $\text{pH} = 6.37$ . If these two extremes be omitted from consideration, the first seven of the collaborators reported observations which varied less than 0.1 in terms of pH.

In view of the characteristics of these samples it was anticipated that Sample No. 2 would have the highest hydrogen-ion concentration because of the treatment with chlorine to which it had been subjected, Sample No. 1 should be intermediate in this particular, while Sample No. 3 should have the lowest hydrogen-ion concentration of the three. All the collaborators reported the three samples in this order of descending hydrogen-ion concentration except two. In one of these two instances (Collaborator No. 8) the findings in the instance of Sample No. 3 were quite evidently too low, and it would appear that some error must have been introduced either in sampling the original lot of flour or at some other point in the handling and determination. In the other instance the same hydrogen-ion concentration is reported for Samples 1 and 3, but none of these determinations was very far out of line when compared with the findings of the other collaborators.

The associate referee is not sufficiently familiar with the use of the quinhydrone electrode to enable him to suggest a reason for the high concentrations of hydrogen-ions which were determined through its use.

This preliminary study will make it possible to recommend a definite method for the determination of the hydrogen-ion concentration of flour which will differ to the least possible extent from the method which is employed in the majority of these laboratories. Thus it would appear that a ratio of 10 grams of flour per 100 cc. of water and an extraction period of 30 minutes with the mixture of flour and water maintained at a temperature of 25°C. would approximate the procedure most commonly in vogue at the present time. It is perhaps unnecessary to specify the concentration of potassium chloride in the calomel half-cell, although the associate referee desires to indicate a distinct preference for the normal potassium chloride electrode with frequent renewal of the chloride solution. From this as a starting point other details should be added by the referee that continues these studies with a view toward recommending a method for collaborative study during the next year.

It is probable that the reason for certain of the variations in these results might be uncovered if the referee should distribute a sample of a buffered solution to each of the collaborators. The Palitzsch borate solution<sup>1</sup> appears to be admirably adapted to such distribution since it remains sterile and is not modified substantially by the solution of glass in contact with it.

It is well known that flour tends to increase in hydrogen-ion concentration with the lapse of time, and it is accordingly suggested that in the next series of collaborative studies it would be well to prescribe the day when the determinations should be made, which will obviate the possibility of a change in this property of flour on storage. A few days either way would not make any appreciable difference in the results.

#### RECOMMENDATIONS<sup>2</sup>.

It is recommended—

(1) That the referee prescribe a method for the collaborative study of the hydrogen-ion concentration of flour based so far as is conveniently possible upon the methods now in use in flour laboratories.

(2) That buffered solutions be distributed to the collaborators, together with samples of flour.

(3) That the collaborators be urged to complete their determinations within a period of five days, in order that the time factor in storage of the flour may not affect the findings.

(4) That the use of the quinhydrone electrode and other electrodes, including the antimony electrode and possibly also the manganese

<sup>1</sup> Clark, W. M. Determination of hydrogen-ions, 1920, p. 83.

<sup>2</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 90.

sesquioxide electrode, be subjected to collaborative study as soon as this can be arranged.

TABLE 1.

*Details of methods and results of determination of the hydrogen-ion concentration of three flour samples.*

COLLABORATOR NO.	QUANTITY OF FLOUR PER 100 CC. OF WATER	TIME OF EXTRACTION	TEMPERATURE OF EXTRACTION	CONCENTRATION OF KCl IN CALOMEL ELECTRODE	H+ TYPE OF ELECTRODE VESSEL	H+ -ION CONCENTRATION AS pH		
						1- Straight Flour Unbleached	2- Straight Flour Treated by Chlorine	3- Clear Flour Unbleached
	grams	minutes	°C.			pH	pH	pH
1	20	60	Room	1.0 N	Bailey	6.20	5.99	6.27
2	10	60	Room	1.0 N	Bailey	6.14	5.95	6.20
3	10	30	25	Saturated	Hildebrand	6.12	5.93	6.22
4	10	30	0-3	Saturated	Bailey	6.12	5.81	6.22
5	10	30	25	1.0 N	Bailey	6.18	5.86	6.18
6	10	30	25	Saturated	Bailey	6.19	5.94	6.25
7	20	60	22	1.0 N	Bailey	6.11	5.92	6.22
8	10	180	25	Saturated	?	6.14	5.94	6.07
9	15	10	Room	Saturated	Hildebrand	6.26	6.08	6.37
10	20	30	Room		Quinhydrone	5.92	5.70	6.04
11	20	30	Room		Quinhydrone	5.89	5.68	6.01

No report on starch and the diastatic value of flour was given by the associate referee.

No report on gluten in flour was given by the associate referee.

## REPORT ON SPECIFIC GRAVITY AND ALCOHOL.

By RAYMOND M. HANN (Bureau of Chemistry, Washington, D. C.),  
*Referee.*

Criticism was directed toward the alcohol and specific gravity tables that appeared in the 1920 edition of *Methods of Analysis* for the following reasons: (1) The temperature used is 20°C. instead of the 15° or 15.56°C. used in all other English speaking countries; (2) the use of water at its point of maximum density as a standard of comparison; (3) failure to include in the directions for the determination of alcohol some mention of the means of obtaining weights "in vacuo" since the tables are based on true and not apparent specific gravities; and (4) volume percentages are calculated for the standard temperature, while those commonly in use are calculated from the 15° or 15.56°C. standard temperatures<sup>1</sup>.

<sup>1</sup> Lyon. *J. Am Pharm. Assoc.*, 1921, 10: 11.

In considering the accurate determination of the specific gravity of a liquid there enter a number of factors that are not ordinarily considered by the chemist. The most exact determination of density<sup>1</sup> of a liquid is made by weighing a suitable sinker in vacuo and again when immersed in the liquid under consideration. The value of the density of the liquid then may be calculated as follows:

$$D \frac{t}{4^{\circ}} = S - \frac{W_1 - w + W_2 - w}{2} \left(1 - \frac{P}{8.4}\right),$$

where  $D \frac{t}{4}$  = density of liquid at temperature  $t$ ,

$S$  = mass of sinker,

$Vt$  = volume of sinker at temperature  $t$ ,

$t$  = temperature of determination,

$W_1, W_2$  = balance readings with sinker on,

$w$  = balance readings with sinker off, and

$P$  = air density.

The term  $\left(1 - \frac{P}{8.4}\right)$  is the buoyancy factor due to the air displaced

by the weights. This correction may be obtained by multiplying the observed air density by the volume of the weights (8.4 in the case of brass). A buoyancy balance may be used directly when available.

In the case of alcohol it is a comparatively simple matter to make such determinations, and the basis of the ordinary alcometric tables is determined in this manner. Careful study has given an accurate value for the thermal expansion of ethyl alcohol solutions, and by means of the formula

$$D \frac{t}{4^{\circ}} = D \frac{20^{\circ}}{4^{\circ}} + (20^{\circ} - t^{\circ}) 0.000846$$

values of any solution over quite a range of temperature may be determined. Comparable results are obtained by use of the formula

$$D \frac{t}{4^{\circ}} = D \frac{25^{\circ}}{4^{\circ}} - \left[ 859(t-25) + 0.6(t-25)^2 + 0.005(t-25)^3 \right] \times 10^{-6},$$

which is derived by the method of least squares on the assumption that the change in density depends upon a change in temperature alone.

Application of such formulas as these is rather time consuming, and for routine determinations a simpler relationship is desirable. In the absence of a buoyancy balance, correction of apparent weight to weight

<sup>1</sup> Specific gravity is the ratio of the density of the substance at  $t^{\circ}$  to the density of water at  $T^{\circ}$ , and when  $T^{\circ} = 4^{\circ}\text{C}$ . the density is equivalent to density expressed in grams per millimeter.

"in vacuo" is essential, and it was for this purpose that the formula about to be considered was introduced by A. E. Paul and tested by R. F. Jackson and the present referee.

The correction to "in vacuo" is employed to compensate for the air displacement factor of the pycnometer and the weights. This is determined by adding to the apparent weight in air a buoyancy correction equal to the weight of the air displaced by the difference in volume of the body weighed and the weights required to balance it on an equal arm balance. Assuming  $W$  is the apparent weight in air,  $P$  is the density of air,  $d^1$  is the density of the body and  $d^2$  is the density of the weights, the value of  $M$ , the weight "in vacuo" is—

$$\begin{aligned} M &= W + P \left( \frac{M}{d^1} - \frac{W}{d^2} \right) = W \frac{d^1}{d^2} \left( \frac{d^2 - P}{d^1 - P} \right) \\ &= W \frac{d^1}{d^1 - P} \left( 1 - \frac{P}{d^2} \right) = W \left[ 1 + \frac{P}{d^2} \left( \frac{d^2 - d^1}{d^1 - P} \right) \right]. \end{aligned}$$

This formula gives exact results when the various quantities involved in its derivation are exactly known. These values vary, of course, with geographic location, with the metal of which the weights are made, and with the density of the body.

Even this formula is somewhat involved, and for ordinary purposes a simpler approximation of the vacuum weight would be of more service. For practical consideration a buoyancy factor may be added to the observed weight and a value very close to the true value obtained. This factor may be calculated

$$B = P (V - Vw),$$

where  $B$  = air buoyancy,

$P$  = air density,

$V$  = volume of water, and

$Vw$  = volume of weights.

Assuming the volume to be equal to the apparent weight of water at 20° (which, however, is not exactly true) the following formulas are obtained:

$$\begin{aligned} B &= P \left( W - \frac{W}{8.4} \right) \\ &= 0.0012 \left( W - \frac{W}{8.4} \right) \\ &= 0.00105 W. \end{aligned}$$

This value is to be added to both numerator and denominator to give the corrected result. Hence the complete operation—



$$M = \frac{S + B}{(1.00177 \times W) + B} = \frac{S + 0.00105 W}{1.00177 W + 0.00105 W}$$

$$= \frac{S + 0.00105 W}{1.00282 W}$$

This formula has been applied to various alcohol and sugar solutions and found to be very nearly correct. It is necessarily somewhat inaccurate since it assumes that the volume of water at 20°C. is the same as its mass, and that at any given altitude the volume of air displaced by the weights is the same for all solutions as it is for water.

The following results were obtained by a study of 50 per cent sugar and alcohol solution:

PER CENT	TRUE	APPARENT
50 sugar	1.22957	1.22962
70 sugar	1.34717	1.34723
50 alcohol	0.91384	0.91384

The simplicity of the formula justifies its use in *Methods of Analysis*, 1925, and it is accordingly recommended.

#### RECOMMENDATIONS.<sup>1</sup>

It is recommended—

(1) That the formula employed for the approximate correction to weight "in vacuo" be adopted as official.

(2) That study be undertaken of the correlation of refractometric and pycnometric methods for the determination of alcohol.

#### REPORT ON VINEGARS.

By J. O. CLARKE<sup>2</sup> (U. S. Food and Drug Inspection Station, Savannah, Ga.), *Referee*.

No report was submitted by the Referee on Vinegars last year. The committee continued from the previous year the recommendation that the method for polarization using decolorizing carbon be further studied<sup>3</sup>.

The referee has reviewed the records of the association since 1909 and finds that comparatively little collaborative work on vinegar has been done. Many of the present methods have been adopted from time to time with little collaborative work. Their adoption was probably governed by the fact that they were in general use and considered reliable. Since the testing of methods through collaborative work is one of the fundamental principles of the association, it was felt that collaborative work should be done on the essential determinations.

<sup>1</sup> For report of Sub-Committee B and action of the association, see *This Journal*, 1926, 9: 75.

<sup>2</sup> Present address: U. S. Food and Drug Inspection Station, New York, N. Y.

<sup>3</sup> *This Journal*, 1924, 7: 272.

The referee for 1923 proposed and recommended for adoption official methods for reducing substances before and after inversion, non-volatile reducing substances, and sulfates. These methods were therefore submitted to collaborative examination.

Two samples of cider vinegar were submitted to collaborators, both undiluted. Sample A was submitted to collaborators unaltered, and Sample B had added to it 12 mg. of sulfur trioxide. Collaborators were requested to follow the methods as printed in *Methods of Analysis, A. O. A. C.*, 1925, 325, the following determinations to be made on Sample A:

- 3<sup>1</sup>. Specific gravity
4. Solids
5. Ash—report results by method (a) and (b)
6. Soluble and insoluble ash
7. Alkalinity of the soluble ash
8. Soluble phosphoric acid. (Advise whether gravimetric or volumetric determination of phosphoric acid was made. If time permits, report by both methods.)
10. Total acids
11. Non-volatile acids
12. Volatile acids
13. Total reducing substances before inversion
14. Total reducing substances after inversion
15. Non-volatile reducing substances (sugar)
16. Volatile reducing substances
17. Alcohol
18. Glycerol
21. Color removed by fullers' earth
22. Lead precipitate
23. Polarization
24. Sulfates

Sample B was designed to obtain additional collaborative results on certain methods, and the following determinations were requested:

13. Total reducing substances before inversion
14. Total reducing substances after inversion
15. Non-volatile reducing substances (sugar)
16. Volatile reducing substances
23. Polarization
24. Sulfates

The referee wishes to express his appreciation to the heads of the collaborating laboratories and to the following chemists who did the analytical work and submitted reports:

1. H. R. Smith, U. S. Food and Drug Inspection Station, Baltimore, Md.
2. L. Katz, U. S. Food and Drug Inspection Station, New York, N. Y.
3. W. H. Heath, U. S. Food and Drug Inspection Station, Buffalo, N. Y.
4. H. J. Fisher, Connecticut Agricultural Experiment Station, New Haven, Conn.

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<sup>1</sup> Numbers refer to paragraphs.

TABLE  
*Collaborative results*  
 (Expressed as grams per 100

DETERMINATIONS	SMITH	KATZ	HEATH
			SAMPLE
Specific gravity 20°/4°C. . . . .	1.0133 <sup>a</sup>	1.013 <sup>a</sup>	1.0134 <sup>a</sup>
Total solids. . . . .	1.79-1.77	1.75-1.75	1.78-1.79
Total ash (Method A). . . . .	0.27	0.28-0.28	0.36-0.36
Total ash (Method B). . . . .	0.27	0.28-0.28	0.32-0.32
Water-insoluble ash. . . . .	0.04-0.04	0.04-0.04	0.04-0.05
Water-soluble ash. . . . .	0.22-0.22	0.24-0.24	0.28-0.27
Alkalinity water-soluble ash (cc. 0.1 N per 100 cc.) . . . . .	26.8-26.4	27.4-27.4	28.7-28.3
Water-soluble phosphoric acid (P <sub>2</sub> O <sub>5</sub> ) (mg. per 100 cc.) . . . . .	3.8-4.7	3.15-3.5	7.1p-5.5p
Total acid as acetic . . . . .	5.62-5.62	5.67-5.67	5.59
Non-volatile acid as acetic. . . . .	0.02-0.02	0.04-0.04	0.05
Volatile acid as acetic. . . . .	5.60-5.60	5.63-5.63	5.54
Total reducing substances before inversion . . . . .	0.44-0.44	0.45-0.46	0.46-0.47
Total reducing substances after inversion. . . . .	0.41-0.43	0.46-0.47	0.47-0.47
Non-volatile reducing substances. . . . .	0.30-0.31	0.30-0.30	0.34-0.33
Volatile reducing substances. . . . .	0.13	0.15-0.16	0.13
Alcohol. . . . .	0.08-0.09	0.08-0.07	0.27
Glycerol. . . . .	0.21-0.20	0.25-0.25	0.27-0.27 <sup>d</sup>
Color removed by fullers' earth (per cent). . . . .	50	51-51 <sup>b</sup>	45
Lead precipitate. . . . .	Normal	Heavy <sup>e</sup>	Normal
Polarization (°V. 200 mm.) . . . . .	-0.5 -0.5	-0.5 -0.5	-0.56
Sulfates (mg. SO <sub>3</sub> per 100 cc.) . . . . .	3.3 3.5	3.7 3.6	3.57 3.53
			SAMPLE
Total reducing substances before inversion . . . . .	0.62-0.62	0.63-0.62	0.59-0.61
Total reducing substances after inversion . . . . .	0.61	0.65-0.64	0.59-0.58
Non-volatile reducing substances. . . . .	0.43-0.43	0.43-0.43	0.42-0.41
Volatile reducing substances. . . . .	0.18	0.21-0.20	0.18
Polarization (°V. 200 mm. tube) . . . . .	-0.5	-0.5 -0.5	-1.5
Sulfates (Mg. SO <sub>3</sub> per 100 cc.) . . . . .	16.8-13.8	18.7	14.6-15.4

<sup>a</sup> Duplicate identical.

<sup>b</sup> The reagent removes 97 per cent of added caramel color from 4 per cent acetic acid solution.

<sup>c</sup> After settling for one-half hour the sediment occupies a volume of 0.8 cc., or 8 per cent of original volume of vinegar.

<sup>d</sup> Four determinations carried to third place, 0.266-0.266-0.269-0.269.

<sup>e</sup> Round bottom platinum dish was used. Not included in summary since round bottom dishes were used.

1.

*on Vinegar.*

cc. unless otherwise stated.)

FISHER	ROE	HICKEY	SALINGER	CALLAWAY	MAXIMUM	MINIMUM
<b>A</b>						
1.0137	1.0132	1.0127	1.0135	1.0134	1.0133	1.0127
1.64 <sup>a</sup> -1.75 <sup>a</sup>	1.67-1.75	1.73-1.74	1.75-1.73	1.76-1.77	1.79	1.67
0.27-0.28	0.31	0.27-0.27	0.30-0.30		0.36	0.27
	0.31-0.31	0.27-0.27		0.30-0.30	0.32	0.27
0.04-0.04	0.05-0.05	0.04-0.04	0.06-0.05	0.04-0.04	0.06	0.04
0.24	0.26-0.26	0.23-0.24	0.24-0.25	0.26-0.26	0.28	0.22
25.78 <sup>a</sup>	28.0-28.8	28.4-28.6	28.0-28.4	28.4-28.4	28.8	25.78
3.4 <sup>b</sup>	4.96-5.08	5.47 <sup>k</sup> -3.64 <sup>l</sup>	2.8-2.9	6.6 6.6	7.1	2.8
5.61-5.62	5.60-5.61	5.63	5.66-5.68	5.64-5.65	5.68	5.59
0.03-0.03	0.07	0.05	0.03-0.03	0.04-0.04	0.07	0.02
5.57	5.53-5.54	5.58	5.63-5.65	5.60-5.61	5.65	5.58
0.45-0.47	0.48-0.48	0.46-0.46	0.45-0.46	0.46-0.46	0.48	0.44
0.46-0.47	0.47-0.45	0.45-0.48	0.47-0.48	0.47-0.47	0.48	0.41
0.30-0.25	0.29-0.30	0.30-0.31	0.31-0.32	0.30-0.31	0.34	0.25
0.19	0.18	0.15-0.16	0.15-0.15	0.16-0.17	0.19	0.13
0.16	0.35-0.28	0.15	None	0.13	0.27	0.00
0.24-0.20	0.27-0.27	0.29-0.28	0.22-0.23	0.22-0.22	0.29	0.20
42	31	24.3	40	50	51	24
Normal	Normal	Light	Medium	Light	Heavy	Light
-0.3	-0.4	-0.4	-0.8	-0.3	-0.8	-0.3
3.1-2.9	4.18	1.82-2.84	3.2-3.1	2.7-2.6	4.18	1.82
<b>B</b>						
0.62	0.62-0.62	0.62-0.61	0.63-0.63	0.62-0.62	0.63	0.59
0.60	0.65-0.67	0.62-0.61	0.67-0.67	0.65-0.64	0.67	0.58
0.26	0.33	0.46-0.48	0.45-0.45	0.42-0.42	0.48	0.26
0.35	0.31	0.16-0.14	0.18-0.18	0.21-0.21	0.35	0.14
-0.13	-0.8	-0.2	-1.2	-0.5	-1.5	-0.13
17.4-16.7	15.9-16.4	14.4-16.0	16.1-17.0	17.9-18.0	18.7	13.8

<sup>f</sup> It was intended to run ash determinations by both methods 5 (a) and 5 (b), but as residue after being left in the muffle below red heat for about 40 hours contained no carbon, this was not done. The difference between the methods seems too slight to warrant classifying them as different methods

<sup>g</sup> Average of four determinations, 25.56-26.96-25.76-24.84.

<sup>h</sup> Average of four determinations, 2.6-2.6-4.1-4.4. Gravimetric method used.

<sup>k</sup> Average 5.75 and 5.19. Ash from Method (a), volumetric method.

<sup>l</sup> Average 3.71 and 3.58. Ash from method (b), volumetric method.

<sup>p</sup> Gravimetric or volumetric method not stated.

5. R. S. Roe, U. S. Food and Drug Inspection Station, Chicago, Ill.
6. C. H. Hickey, U. S. Food and Drug Inspection Station, Boston, Mass.
7. L. A. Salinger, U. S. Food and Drug Inspection Station, Savannah, Ga.
8. Joseph Callaway, Jr., U. S. Food and Drug Inspection Station, Savannah, Ga.

## COMMENTS BY COLLABORATORS.

*H. R. Smith:*

*Specific gravity:* There seems to be no object in the determination of specific gravity, as the other determinations meet the requirements more satisfactorily.

*Total ash:* Method (a) is preferred as being more direct and free from uncertainty and requiring less time of the analyst.

*Polarization:* The polarization was difficult and the results unsatisfactory. There does not seem to be sufficient value to this determination to warrant its retention in the methods for vinegar analysis.

*H. J. Fisher:*

*Glycerol:* It is suggested that in the glycerine determination the possibility of substituting the method of Knop<sup>1</sup> for the ferricyanide method in the final titration might be investigated. Outside indicators are to be avoided if possible. Experiments in standardizing the ferrous ammonium sulfate solution by both methods showed 1 cc. of potassium dichromate (dilute) equivalent to 1.002 cc. of ferrous ammonium sulfate by the Knop method as against 1.014 cc. by the ferricyanide method.

## DISCUSSION.

Collaborative results on the following official methods show an acceptable agreement, and further study is not necessary: specific gravity, total solids, insoluble ash, alkalinity of water-soluble ash, total acid, non-volatile acid, volatile acid, and alcohol. Acceptable results on the tentative methods for reducing substances before and after inversion were obtained.

Erratic results for total ash indicate the necessity for further work. Closer agreement is found with Method (b). Using Method (a) the spread between maximum and minimum results is too great for an important determination of this character. Results for water-insoluble ash are satisfactory. Water-soluble ash naturally follows total ash and should be the subject of further study.

The very erratic results for water-soluble phosphoric acid are difficult to explain, and this method should be studied. Perhaps the trouble is in the distribution of water-soluble and water-insoluble phosphoric acid produced by variations in the ash method rather than in the actual determination of phosphoric acid. This point should be studied. It may be necessary to require a determination of total phosphoric acid rather than both water-soluble and water-insoluble phosphoric acid.

A wide variation in non-volatile reducing substances and consequently in volatile reducing substances is shown in both Sample A and Sample B. Since good results are reported on total reducing substances, the trouble

<sup>1</sup> J. Am. Chem. Soc., 1924, 46: 264.

is possibly in the treatment previous to the determination of sugar. This method should receive further study before its final adoption as official.

The determination of glycerol is important in vinegar analysis—perhaps the most important of the routine methods in testing the purity of vinegar under regulatory acts. Results varying almost uniformly from 0.20–0.29 gram per 100 cc. were obtained, there being no exceptionally high or low results that might be charged to errors on the part of one or two chemists.

Color removed by fullers' earth is a semi-qualitative test for added caramel color. While the results are not particularly bad, they should be closer. The method should receive further study.

Reports for lead precipitate run from heavy to light. It does not seem advisable to retain so crude a method, especially since it gives no useful information as to the purity of the sample. It would seem wise, therefore, to drop this determination.

Polarization is of no particular value in determining the purity of vinegar. It gives no results which are not more accurately determined by more reliable methods. Because the determination does not appear susceptible of greater accuracy than reported, it should be dropped.

Sample A was a generator-run vinegar. Sample B had a quantity of sulfate added to approximate that found in apple waste vinegar. The results are fairly close, but further study is suggested before the method is finally adopted as official.

#### RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That methods for total ash and water-soluble ash be further studied.

(2) That methods for phosphoric acid be studied, the advisability of dropping the present methods for soluble and insoluble phosphoric acid and substituting a method for total phosphoric acid being considered.

(3) That Method 13—Total Reducing Substances Before Inversion—be adopted as official (final action).

(4) That Method 14—Total Reducing Substances After Inversion—be adopted as official (final action).

(5) That further study be given to the following methods: 15—Non-Volatile Reducing Substances; 16—Volatile Reducing Substances; 18—Glycerol; 24—Sulfates.

(6) That Method 22—Lead Precipitate—and 23—Polarization—be dropped.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 86.

## REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES.

By J. W. SALE<sup>1</sup> (Bureau of Chemistry, Washington, D. C.), Referee.

A review of previous reports showed that no work had been done on five recommendations made by former referees, namely, Nos. 1, 2, 3, 7, and 8 as listed in the report of the referee for 1924<sup>2</sup>. The referee wrote to eight chemists who were experienced in the analysis of flavors and asked which of these five recommendations should in their opinion be given preference. As a result of this correspondence Nos. 2 and 7 were selected. They are as follows:

(2) 1916: That the value of the test for the detection of vanilla resin be studied<sup>3</sup>.

(7) 1919-20-21-22: That the referee give consideration to methods for the analysis of non-alcoholic flavors, as for example the determination of orange oil and lemon oil in mineral oil, cottonseed oil, etc.<sup>4</sup>

## DETERMINATION OF RESIN IN VANILLA EXTRACTS.

There is a wide difference of opinion as to the value of the gravimetric determination of vanilla resin. Hess<sup>5</sup>, who developed the qualitative tests for vanilla resin, did not specifically direct the separation and weighing of the resin but merely stated: "If it is desirous to weigh the resin for a quantitative determination, several hours are necessary for its complete separation". Brooks<sup>6</sup>, however, described a gravimetric procedure. One experienced chemist advised in his correspondence that the tests for vanilla resin are too indefinite and mean nothing, while a representative of a commercial laboratory stated that the gravimetric determination was of great value to him in evaluating vanilla extracts.

The two procedures described below were selected for collaborative work:

## METHOD I.

Transfer 50 cc. of the extract to a 250 cc. beaker. Add 25 cc. of water and evaporate to 30 cc. Add 20 cc. of water and make the solution slightly acid with acetic acid, cover, and let stand overnight. Filter and wash with water by decantation, reserving the filtrate. Dissolve the resins on the filter paper and in the beaker with a small quantity of 95 per cent alcohol. Collect the solution in a weighed beaker, evaporate, and dry at 100°C. for 1 hour. Weigh, dry for another hour, and reweigh. Calculate to grams per 100 cc.

## METHOD II.

*Quantitative Test—Tentative.*

Pipet 50 cc. of the extract into a small beaker, add 50 cc. of water, evaporate to 50 cc. on the steam bath, add 50 cc. of water, again evaporate to 50 cc., and cool; if the mixture has an acid reaction, add 1 cc. of hydrochloric acid (1 + 1). If the mixture is not acid to litmus, add hydrochloric acid (1 + 1) drop by drop until distinctly acid to

<sup>1</sup> Presented by J. B. Wilson.

<sup>2</sup> *This Journal*, 1925, 8: 686.

<sup>3</sup> *Ibid.*, 1920, 3: 533.

<sup>4</sup> *Ibid.*, 1921, 4: 479, 580, etc.

<sup>5</sup> *J. Am. Chem. Soc.*, 1899, 21: 719

<sup>6</sup> *Am. Perfumer*, 1908, 3: 167.

TABLE 1.  
*Gravimetric determination of resins in authentic extracts of standard strength.*

EXPERI- MENT NO.	SAMPLE NO.	COLLABORATOR	VARIETY OF VANILLA BEAN	ALCOHOL, BY VOLUME	INTERFERING SUBSTANCES	ACIDU- LENT	WEIGHT OF RESINS WHEN DILUTED AS INDICATED*					
							per cent	per cent	per cent	per cent	per cent	per cent
1	1	C. E. G.†	Mexican Bourbon	60.6	None	AcOH	0.119	0.104	0.082	0.030	0.007	0.003
2	2	"	"	47.5	"	"	0.111	0.068	0.026	0.027	0.014	0.002
3	3	C. H. B.‡	"	60.6	"	"	0.069	0.046	0.023	0.008	0.003	0.002
4	3	C. E. G.	South American	47.5	"	"	0.080	0.036	0.012	0.008	0.005	0.003
5	4	"	"	47.5	"	"	0.069	0.044	0.020	0.013	0.004	0.001
6	4	C. H. B.	"	47.5	"	"	0.069	0.063	0.026	0.013	0.008	0.003
7	4	"	"	47.5	"	"	0.069	0.063	0.026	0.013	0.008	0.003
8	5	C. E. G.	Tahiti Vanillons	60.3	"	"	0.066	0.041	0.018	0.004	0.002	0.000
9	6	"	"	47.5	"	"	0.093	0.064	0.026	0.011	0.005	0.001
10	6	C. H. B.	"	47.5	"	"	0.074	0.033	0.016	0.009	0.003	0.001
11	3	C. E. G.	Bourbon	47.5	"	HCl	0.090	0.030	0.016	0.009	0.006	0.002
12	3	C. H. B.	"	47.5	"	"	0.116	0.083	0.046	0.022	0.013	0.005
13	4	C. E. G.	South American	47.5	"	"	0.110	0.093	0.041	0.023	0.009	0.003
14	4	C. H. B.	"	47.5	"	"	0.096	0.113	0.056	0.026	0.013	0.004
15	4	"	"	47.5	"	"	0.114	0.114	0.059	0.028	0.015	0.004
16	6	C. E. G.	Vanillons	47.5	"	"	0.106	0.064	0.025	0.009	0.003	0.001
17	6	C. H. B.	"	47.5	"	"	0.085	0.084	0.050	0.025	0.013	0.006
18	8	C. E. G.	Mexican Bourbon	62.6	0.6 K <sub>2</sub> CO <sub>3</sub>	AcOH	0.136	0.105	0.040	0.007	0.003	0.000
19	7	"	"	61.3	0.6 K <sub>2</sub> CO <sub>3</sub>	"	0.125	0.089	0.022	0.010	0.004	0.003
20	9	"	"	47.5	0.1 NH <sub>3</sub>	"	0.119	0.104	0.046	0.022	0.007	0.000
21	10	"	"	47.5	0.2 NH <sub>3</sub>	"	0.116	0.086	0.040	0.020	0.009	0.001
22	11	"	South American	47.5	0.1 NH <sub>3</sub>	"	0.090	0.063	0.033	0.024	0.007	0.003
23	12	"	"	47.5	0.2 NH <sub>3</sub>	"	0.104	0.066	0.038	0.021	0.008	0.004
24	13	"	Vanillons	47.5	0.1 NH <sub>3</sub>	"	0.154	0.112	0.056	0.029	0.015	0.001
25	14	"	"	47.5	0.2 NH <sub>3</sub>	"	0.229	0.176	0.086	0.040	0.020	0.003
26	15	"	Tahiti Vanillons	60.9	0.6 K <sub>2</sub> CO <sub>3</sub>	"	0.113	0.096	0.043	0.012	0.008	0.002
27	14	"	South American	47.5	0.2 NH <sub>3</sub>	HCl	0.238	0.096				
28	15	"	"	41.7	20 Sugar	AcOH	0.096					
29	16	"	"	41.0	20 Glycerol	"	0.084					
30	17	"	Mexican	59.4	2 Caramel	"	0.088					
31	18	"	"	47.5	1 Vanillin	"	0.102					

\* 100 per cent represents the undiluted standard extract; 80 per cent represents the extract diluted from 80 cc. to 100 cc. with water, etc.

† Charles E. Goodrich.

‡ Cecil H. Badger.



litmus paper, then 1 cc. in excess. Cover and let stand overnight. Filter, and wash 6 or 7 times with approximately 0.05 *N* hydrochloric acid, 9 cc. of hydrochloric acid (1+1) per liter of water. Dissolve the resin in warm 95 per cent alcohol by pouring through the filter. Evaporate the alcohol in a tared 50 cc. beaker and dry to constant weight at 100°C. Reserve the resin for qualitative tests.

In Method I, which is based on Brooks' description, acetic acid is employed as an acidulent, and the solution is evaporated once from 75 cc. to 30 cc. to remove alcohol, while in Method II, which was obtained from a commercial laboratory where it is regularly used, the acidulent is hydrochloric acid, and the directions call for two evaporations to remove the alcohol. Moreover, the procedure in Method II is more specific than in Method I. Hereafter, these methods will be referred to briefly as the acetic acid method and the hydrochloric acid method, respectively.

The two procedures described were applied to 18 standard extracts of varying concentrations in the Water and Beverage Laboratory, Bureau of Chemistry. The following varieties of beans were represented in the extracts: Mexican, Bourbon, South American, Tahiti, and Vanillons. Two collaborators, Cecil H. Badger and Charles E. Goodrich of the same laboratory, conducted the tests, the results of which are given in Table 1.

#### DISCUSSION OF DATA IN TABLE 1.

The data in Table 1 show that the weights of resin are not strictly proportional in any case to the percentage of true vanilla extract that is present, but that generally speaking they are approximately proportional in all dilutions except 1, 5, and 10 per cent. More resin is generally obtained in the 10 per cent solutions than in the 5 per cent and more in the 5 per cent than in the 1 per cent solutions. A number of exceptions, however, occur in all dilutions, and it is obvious that the weight of resin should not be employed to calculate, in exact terms, the percentage of true vanilla.

There is considerable disagreement in some cases in the determinations made on the same sample by different analysts, as for example in Experiments 11 and 12. Usually, however, the agreement is fair as in Experiments 5 and 6 and in 13 and 14.

The weight of resin in extracts made from vanillons (*vanilla pompona*), Experiments 9, 10, 16, 17, is about the same as in true vanilla extracts (*vanilla planifolia*).

The weight of resin in extracts made by the use of alkali is higher than in those made without alkali, as shown in Experiments 18 to 27, inclusive.

The addition of sugar, glycerol, caramel, and vanillin in substantial quantities does not materially affect the results, as shown in Experiments 28 to 31, inclusive.

The acetic acid method and the hydrochloric acid method appear to give similar results, which, however, are more consistent when obtained by the hydrochloric acid method. Both collaborators reported that the solutions of resin filtered more rapidly when the hydrochloric acid method was used and that this method gave clearer filtrates.

It is the opinion of the referee that neither of the methods is worthy of adoption by the association as official, because the weights of resin obtained on the same solution vary considerably. The question as to whether or not the hydrochloric acid method, which is the more satisfactory method, should be adopted as a tentative method has been carefully considered. It is believed that it is valuable provided the results are not interpreted too literally, and provided no attempt is made to calculate the exact percentage of true vanilla from the weights of resin. The limitations of the method in this particular can be indicated by requiring that the weights of resin be reported in grams per 100 cc. to two decimal places only. With this modification, it is recommended that the method be adopted as a tentative method.

The data in Table 1 also indicate that the normal quantity of resin in a standard vanilla extract or extract of vanillon, made without the use of alkali, is about 0.10 per cent as determined by the hydrochloric acid method. When alkali is employed the resin obtained is increased, especially in extract of vanillons. It may be stated that these figures do not agree with those reported by Leach<sup>1</sup> for resin in vanilla beans, namely, 4–11 per cent. In this connection the referee agrees with Brooks when he states that the proportion of resin present in vanilla beans has been grossly overstated. Brooks found that in 18 "pure, high grade" extracts examined by him, the resin content ranged from 0.096–0.47 per cent, only two of the samples showing over 0.2 per cent. The following percentages of resin were obtained by him on authentic extracts: 0.128 per cent (Mexican whole beans), 0.196 per cent (Mexican cut beans), 0.192 per cent (Seychelle bourbon cured), 0.180 per cent (Comoros Nossi Bey), 0.188 per cent (Comoros short). He stated that these results indicate that a 0.1 per cent minimum limit for resin would be liberal enough for all purposes. At the 1909 meeting of the A. O. A. C., the Associate Referee on Colors reported the following results on vanilla resin<sup>2</sup>: 0.034 (50 per cent by volume true vanilla extract), 0.027 (50 per cent by volume true vanilla extract), 0.071 (true vanilla extract with 50 per cent alcohol) all results in grams per 100 cc. A representative of a commercial laboratory advised that his data indicated that the normal weight of resin for standard vanilla extracts varied from 0.08–0.11 gram per 100 cc.

<sup>1</sup>Food Inspection and Analysis, 4th ed., p. 919.

<sup>2</sup>Bur. Chem. Bull. 132, p. 57.

## QUALITATIVE TESTS FOR RESIN IN VANILLA EXTRACT.

Four qualitative tests for vanilla resin requiring the following reagents: 5 per cent potassium hydroxide solution, ferric chloride, and strong hydrochloric acid, basic lead acetate solution, and gelatine solution, are described in the official methods<sup>1</sup>. These tests were applied in the manner described to the dried resins, the weights of which are recorded in Table 1 and reactions characteristic of true vanilla resins were obtained with all reagents except that ferrous chloride gave a greenish coloration owing to a reaction with the 95 per cent alcohol that was used to dissolve the resin.

As foreign resins, such as resin from St. John's bread, are sometimes used to adulterate vanilla extract, the tests were applied to the resin obtained from the following commercial oleoresins: Aspidii, capsicum, ginger, cubeb, and black pepper. The tests were also applied to balsam tolu and St. John's bread. The oleoresins and powdered balsam tolu were digested in 95 per cent alcohol, a quantity of magnesium carbonate was added, and the solutions were diluted gradually with an equal volume of water while stirring. The resulting solutions were filtered until clear or nearly so and dealcoholized by the addition of water followed by evaporation on the steam bath. An extract of St. John's bread was made by using the powdered bean and 47½ per cent alcohol. The tests were applied to the material, which was filtered out after dealcoholizing the clear 47½ per cent alcohol solutions. The results are given in Table 2, together with the results on true vanilla resin, which are included for comparison.

The results on foreign resins, Table 2, show that the qualitative tests are useful in differentiating foreign resins from vanilla resin, and it is believed that they should be retained to supplement the gravimetric determination by the hydrochloric acid method.

## ANALYSIS OF NON-ALCOHOLIC FLAVORS.

In addition to the work on resins, methods for the analysis of non-alcoholic flavors were studied. Thirty solutions of lemon oil and of orange oil in corn oil, cottonseed oil, and light mineral oil were prepared, the percentages by volume of essential oil in each type of solution being as follows: 1.67, 3.33, 5.00, 6.67 and 8.33. The essential oils had been manufactured from California fruit. The samples were analyzed by a steam distillation method and also by determining their rotation in the polariscope by Goodrich and by J. B. Wilson, of the Water and Beverage Laboratory, Bureau of Chemistry.

*Steam Distillation Method.*

The samples were subjected to steam distillation, 200 cc. of distillate

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 351.

TABLE 2.  
*Qualitative tests on foreign resins\*.*

NAMES OF COMMERCIAL OLEORESIN OR BALSAM	APPEARANCE OF RESIN ON FILTER	ACTION WITH REAGENTS†					
		5% KOH solution	On acidifying	F Cl <sub>3</sub> solution	Strong HCl	Basic lead acetate solution (filtrate)	Gelatin (filtrate)
Aspidii	reddish brown; part salmon color	soluble dark brown	brown precipitate flocculent	brown precipitate	brown precipitate	white precipitate	no change
Capsicum	brown	soluble brown	slight precipitate yellow	no precipitate yellow color	yellow precipitate	bulky white precipitate	"
Ginger	reddish brown	soluble brown	light brown precipitate	no precipitate brown color	brown precipitate	"	"
Cubeb	tree-green	Insoluble		no change	pink precipitate	"	"
Black pepper	olive green	"		green color	no change	"	"
Balsam tolu	reddish brown	soluble red	gelatinous white precipitate	green precipitate	bulky white precipitate	"	"
St. John's bread	light brown	soluble brownish red	reddish precipitate flocculent	heavy dark green precipitate	flocculent precipitate	granular	heavy precipitate
Vanilla	red flocculent	soluble deep red	red precipitate flocculent	no change	no change	granular (flaky)	turbid

\* Experimental work conducted by C. E. Goodrich.

† *Methods of Analysis*, A. O. A. C., 1925, p. 351.

TABLE 3.

*Determination of essential oils in non-alcoholic flavors by steam distillation.*

EXPERIMENT NO.	COLLABORATOR	ESSENTIAL OIL PRESENT, BY VOLUME	ESSENTIAL OIL RECOVERED, BY VOLUME	CORRECTED FIGURE	
				FOR ESSENTIAL OIL FOUND*	ERROR
		<i>per cent</i>	<i>per cent</i>		<i>per cent</i>
Quantity taken§: 50 cc. lemon oil; apparatus: trap; menstruum: corn oil					
1	C. E. G.†	1.67	1.4	1.5	-10
2	"	3.33	2.6	2.7	-19
3	"	5.00	4.4	4.6	-8
4	"	6.67	6.0	6.3	-6
5	"	8.33	7.4	7.8	-6
Quantity taken§: 100 cc. lemon oil; apparatus: tube; menstruum: corn oil.					
6	C. E. G.	1.67	1.5	1.6	-4
7	"	3.33	3.1	3.3	-1
8	"	5.00	4.6	4.8	-4
9	"	6.67	6.2	6.5	-2
10	"	8.33	7.6	8.0	-4
11	"	1.67	1.5	1.6	-4
12	"	3.33	3.1	3.3	-1
13	"	5.00	4.6	4.8	-4
14	"	8.33	7.6	8.0	-4
15	J. B. W.‡	1.67**	1.6	1.7	2
16	"	5.00	4.6	4.8	-4
17	"	8.33	7.6	8.0	-4
Quantity taken§: 100 cc. orange oil, apparatus: tube; menstruum: corn oil.					
18	C. E. G.	1.67	1.6	1.7	2
19	"	3.33	3.2	3.4	2
20	"	5.00	4.6	4.8	-4
21	"	6.67	6.2	6.5	-2
22	"	1.67	1.6	1.7	2
23	J. B. W.	5.00	4.8	5.0	-0
24	"	6.67	6.3	6.6	-1
Quantity taken§: 100 cc. lemon oil; apparatus: tube; menstruum: cottonseed oil.					
25	C. E. G.	1.67	1.5	1.6	-4
26	"	3.33	3.0	3.2	-4
27	"	5.00	4.6	4.8	-4
28	"	6.67	6.2	6.5	-3
29	"	8.33	7.6	8.0	-4
30	J. B. W.	3.33	3.2	3.4	2
31	"	5.00	4.6	4.8	-4
32	"	8.33	7.6	8.0	-4
Quantity taken§: 100 cc. orange oil; apparatus: tube; menstruum: cottonseed oil.					
33	"	1.67	1.6	1.7	2
34	"	5.00	4.8	5.0	-0
35	"	8.33	8.1	8.5	2
Quantity taken§: 100 cc. lemon oil; apparatus: tube; menstruum: mineral oil.					
36	C. E. G.	5.00	4.9	4.8	-4
37	"	8.33	7.9	8.0	-4
38	J. B. W.	1.67	1.8	1.6	-4
39	"	1.67	1.8	1.6	-4
40	"	3.33	3.3	3.2	-4
41	"	5.00	4.9	4.8	-4
42	"	8.33	8.0	8.1	-3
Quantity taken§: 100 cc. orange oil; apparatus: tube; menstruum: mineral oil.					
43	C. E. G.	1.67	1.8	1.6	-4
44	"	5.00	4.9	4.8	-4
45	"	8.33	8.0	8.1	-3
46	J. B. W.	1.67	1.9	1.7	2
47	"	3.33	3.4	3.3	-1
48	"	5.00	4.9	4.8	-4
49	"	6.67	6.6	6.6	-1
50	"	8.33	8.2	8.3	-0

\* Quantity recovered divided by 0.95. Blank for mineral oil 0.3 per cent.

† Charles E. Goodrich.

‡ J. B. Wilson.

§ 200 cc. was distilled in all cases, and essential oils were recovered in a modified Florentine flask.

\*\* Quantity taken: 56 cc.

being collected in 30 minutes in a receptacle of a Florentine flask type, the neck of which consisted of a section of a buret. The volume of recovered oil could be read without further manipulation as soon as the distillation was completed. The receptacle used had a capacity of 250 cc., and the portion of the 50 cc. buret that was sealed to it had a capacity of 20 cc. Smaller flasks with shorter necks were found to be less desirable. A constant level of the distillate was maintained by the siphoning device typical of the Florentine flask. In a few preliminary experiments a Kjeldahl trap was used between the distilling flask and the condenser. The results obtained by the steam distillation method are given in Table 3.

Because the results were excessively low when the Kjeldahl trap was used, it was replaced by a bent glass tube about 8 mm. in diameter. Higher results were then obtained, and no trouble was experienced from the menstruum being carried over mechanically. A distillate amounting to 0.3 per cent by volume was obtained when the mineral oil was subjected to steam distillation, so that this amount was always subtracted as a blank from the volume of oil recovered from those samples having a menstruum of mineral oil. Since, as Boyles<sup>1</sup> points out, only 95 per cent of essential oil is recovered in the case of lemon and orange flavors, the amount actually recovered (corrected for the blank when the menstruum is mineral oil) should be divided by 0.95 to give the corrected result.

#### DISCUSSION OF DATA IN TABLE 3.

The data in Table 3 show that the maximum percentage error in 45 tests in which the glass tube was used, was -4 per cent, the average being 2.9 per cent. The results are therefore satisfactory, and the method seems worthy of further consideration. Before it is offered for adoption, however, tests should be made and peanut oil used as a menstruum. Other specimens of corn oil, cottonseed oil, and mineral oil should be tested as well as other samples of lemon and orange oils and other essential oils. It is desirable also to apply the method to emulsion flavors.

#### *Polariscope Method.*

The rotation of 20 of the authentic samples of flavors referred to was determined at 20.7°-23.0°C., 200 mm. tubes being used. From the readings factors were calculated, with the results shown in Table 4. It is interesting to note that the same factor, namely 5.7, is suitable for orange oil in both cottonseed oil and mineral oil. The other factors, however, are different and depend both upon the kind of essential oil and kind of menstruum which is present. Each of the factors finally selected represents 4 or 5 determinations and 20 or 25 readings. When the factors were applied to the average readings the results set forth in Table 5 were obtained.

<sup>1</sup> *J. Ind. Eng. Chem.*, 1918, 10: 537.

TABLE 4.

*Determination of factors for conversion of polariscope readings to percentage of essential oil\*.*

EXPERIMENT NO.	ESSENTIAL OIL PRESENT, BY VOLUME	POLARISCOPE READING†	POLARISCOPE READING CORRECTED‡	FACTORS§
	per cent	° V.		
Lemon oil; menstruum, corn oil.				
1	0.00	+ 0.6		
2	1.67	+ 5.1	+ 4.5	2.69**
3	3.33	+11.72	+11.12	3.34
4	5.00	+17.35	+16.75	3.35
5	6.67	+24.21	+23.61	3.51
				Average 3.4
Orange oil; menstruum, corn oil.				
1	0.00	+ 0.6		
6	1.67	+ 9.2	+ 8.6	5.15
7	3.33	+18.9	+18.3	5.50
8	5.00	+27.74	+27.14	5.43
9	6.67	+37.3	+36.7	5.50
				Average 5.4
Lemon oil; menstruum, cottonseed oil.				
10	0.00	- 0.3		
11	1.67	+ 5.84	+ 6.14	3.67
12	3.33	+12.0	+12.3	3.09
13	5.00	+18.26	+18.56	3.71
14	6.67	+24.16	+24.46	3.67
15	8.33	+30.9	+31.2	3.74
				Average 3.7
Orange oil; menstruum, cottonseed oil.				
10	0.00	- 0.3		
16	1.67	+ 9.2	+ 9.5	5.69
17	3.33	+18.88	+19.18	5.73
18	5.00	+27.92	+28.22	5.64
19	6.67	+37.5	+37.8	5.67
20	8.33	+46.96	+47.26	5.67
				Average 5.7
Lemon oil††, menstruum, mineral oil.				
21	0.00	+ 5.5		
22	1.67	+11.5	+ 6.1	3.60
23	3.33	+17.4	+12.1	3.58
24	5.00	+23.9	+18.7	3.68
25	6.67	+29.5	+24.4	3.60
26	8.33	+35.0	+30.0	3.54
				Average 3.6
21	0.00	+ 5.5		
27	1.67	+11.6	+ 6.2	3.06
28	3.33	+18.1	+12.8	3.78
29	5.00	+24.3	+19.1	3.76
30	8.33	+36.5	+31.5	3.72
				Average 3.7
Orange oil; menstruum, mineral oil.				
21	0.00	+ 5.5		
31	1.67	+14.88	+ 9.5	5.64
32	3.33	+21.34	+19.0	5.64
33	5.00	+34.02	+28.8	5.70
34	6.67	+43.32	+38.2	5.68
35	8.33	+52.5	+47.5	5.64
				Average 5.7

\* Experimental work conducted by J. B. Wilson

† Each entry is the average of 5 readings made in 200 mm. tubes at 20.7°-23.0°C.

‡ The rotation of the vegetable oils is so small that the correction in all concentrations used consists merely of subtracting the rotation of the menstruum from the rotation of the sample. The rotation of the mineral oil, however, varies with the concentration so that the amount to be subtracted is obtained in each case by multiplying the percentage of mineral oil in the flavor by the rotation of the mineral oil.

§ The factors are obtained by dividing the corrected polariscope reading by the percentage of essential oil present.

\*\* Omitted from average.

†† This set of samples was 6 months old; all others were freshly made.

TABLE 5.

*Determination of essential oils in non-alcoholic flavors by polariscope\*.*

EXPERIMENT NO.	ESSENTIAL OIL PRESENT, BY VOLUME	POLARISCOPE READING†	ESSENTIAL OIL FOUND, BY VOLUME‡	ERROR
	per cent	°V.	per cent	per cent
Lemon oil; menstruum, corn oil.				
1	0.00	+ 0.6		
2	1.67	+ 5.1	1.32	-21.0
3	3.33	+11.7	3.26	- 2.1
4	5.00	+17.4	4.94	- 1.2
5	6.67	+24.2	6.94	+ 4.0
Orange oil; menstruum, corn oil.				
1	0.00	+ 0.6		
6	1.67	+ 9.2	1.59	- 5.0
7	3.33	+18.9	3.39	+ 1.8
8	5.00	+27.7	5.02	+ 0.4
9	6.67	+37.3	6.80	+ 1.9
Lemon oil; menstruum, cottonseed oil.				
10	0.00	- 0.3		
11	1.67	+ 5.8	1.65	- 1.2
12	3.33	+12.0	3.32	- 0.3
13	5.00	+18.3	5.03	+ 0.6
14	6.67	+24.2	6.62	- 0.7
15	8.33	+30.9	8.43	+ 1.2
Orange oil; menstruum, cottonseed oil.				
10	0.00	- 0.3		
16	1.67	+ 9.2	1.67	± 0.0
17	3.33	+18.9	3.37	+ 1.2
18	5.00	+27.9	4.95	- 1.0
19	6.67	+37.5	6.63	- 0.6
20	8.33	+47.0	8.30	- 0.4
Lemon oil; menstruum, mineral oil.				
21	0.00	+ 5.5		
22	1.67	+11.6	1.65	- 1.2
23	3.33	+18.1	3.40	+ 2.1
24	5.00	+24.3	5.08	+ 1.6
25	8.33	+36.5	8.38	+ 0.6
21	0.00	+ 5.5		
26	1.67	+11.5	1.67	± 0.0
27	3.33	+17.4	3.30	- 0.9
28	5.00	+23.9	5.11	+ 2.2
29	6.67	+29.5	6.66	- 0.2
30	8.33	+35.0	8.19	- 1.7
Orange oil; menstruum, mineral oil.				
21	0.00	+ 5.5		
31	1.67	+14.9	1.65	- 1.2
32	3.33	+24.3	3.30	- 0.9
33	5.00	+34.0	5.00	± 0.0
34	6.67	+43.3	6.63	- 0.6
35	8.33	+52.5	8.24	- 1.1

\* All determinations made by J. B. Wilson.

† Each entry is the average of 5 readings taken in 200 mm. tubes at 20.7°-23.0°C.

‡ In the case of the vegetable oils the percentage of essential oil found is obtained by subtracting the rotation due to the menstruum from the reading and dividing the result by the appropriate factor obtained in Table 4. In the case of the mineral oil, however, the rotation due to the menstruum varies with the concentration and the percentage of essential oil found is obtained by means of the following formula:

$$p1 = \frac{rm - r2}{r1 - r2}, \text{ in which } p1 = \text{percentage of essential oil; } rm = \text{rotation of flavor; } r1 = \text{rotation of essential oil; } r2 = \text{rotation of menstruum. (Landolt. Optical Rotation of Organic Substances, 2nd ed. p. 240.)}$$



## DISCUSSION OF DATA IN TABLE 5.

The data in Table 5 show that the maximum percentage error on 31 samples was -5.0 per cent, the average being 1.2 per cent. One obviously abnormal figure, namely -21.0, Experiment 2, was discarded. The results, therefore, are very satisfactory, but additional work along the lines indicated in the discussion of the steam distillation method should be done before the method is offered to the association for adoption.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That final action on the Folin and Denis rapid colorimetric method<sup>2</sup>, described in the referee's report for 1924<sup>3</sup>, be deferred for another year.

(2) That the Wichmann method, for the determination of the lead number of vanilla extract and its imitations, described in the referee's report for 1924, and also in the report on Changes in the Methods of Analysis<sup>4</sup>, be adopted as an alternative official method. (Second presentation; first presented in 1924.)

(3) That the chromate method for the determination of lead, described in the referee's report for 1924<sup>3 4</sup>, be adopted as an alternative official method. (Second presentation; first presented in 1924.)

(4) That the hydrochloric acid method (Method II) for the gravimetric determination of resins in vanilla extracts, described in this report, be adopted as a tentative method when modified by the statement, "Report results to two decimal places only". (First presentation.)

(5) That the statement in *Methods of Analysis*, A. O. A. C., 1925, Chapter XXVII, Page 350, Paragraph 11, beginning "Place 50 cc. of the extract" and ending "filtrate for further tests", be deleted, and that the statement (top of p. 351) "Place a portion of the filter with the attached resins" be changed to "Place a portion of the dried resin". Remove the heading "Qualitative Test—Tentative" from its present position so that it will apply to the qualitative tests.

(6) That the qualitative tests in Chapter XXVII, Paragraph 11, for vanilla resin, be retained.

(7) That the referee for next year continue clearing away old unacted upon recommendations listed in the report of the referee for 1924 and that the work begun this year on the analysis of non-alcoholic flavors be completed.

(8) That additional work be done on the Folin and Denis rapid colorimetric method referred to in Recommendation 1, with a view to determining the effect of added caramel.

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 87.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1912, 4: 670.

<sup>3</sup> *This Journal*, 1925, 8: 686.

<sup>4</sup> *Ibid.*, 1926, 9: 47.

## REPORT ON MEAT AND MEAT PRODUCTS.

By R. H. KERR (Bureau of Animal Industry, Washington, D. C.),  
*Referee.*

No collaborative tests were made as no samples were sent out. Attention was given in the referee's own laboratory to the method for the determination of nitrite in meats adopted as a tentative method at the last meeting. No changes are recommended as a result of the work done, but it appears probable that this method may be successfully modified in some details so as to permit the use of a larger sample, thus reducing the unavoidable variation in results owing to the small samples used in the present tentative method. Before recommending any changes, however, it is believed to be advisable to have some collaborative work done, and it is hoped that during the coming year a sufficient number of collaborators will be available to make such tests.

Consideration was also given to the method for the determination of sugar in meats. Efforts to fill the position of associate referee on this determination have not been successful. For the ensuing year it is recommended that this title be dropped. The present method for sugar in meats is one that was developed as a research method for the determination of muscle sugar and is, therefore, hardly the sort of a method that this association should be concerned with. The requirements of the association are rather for a method for the determination of added sugar used in curing or contained in prepared meat products. Some work along the line of developing an analytical method for sugar has been done in the referee's own laboratory, but no definite results can be reported at present.

No report has been received from W. S. Ritchie, Associate Referee on the Separation of Meat Proteins. Comment on this part of the work, therefore, is reserved.

Attention has been given to the deletion of certain methods which appear to be of doubtful utility or applicability to the needs of this association. Several methods now included in *Methods of Analysis* appear to be intended for research on the composition of meats rather than for the detection of adulteration or such other purposes as directly concern members of this association. Particular examples are the methods for soluble nitrogen, coagulable nitrogen, proteose, peptone, and gelatine nitrogen, amino nitrogen, meat bases, soluble phosphorus, and the separation of soluble inorganic and organic phosphorus. The referee considers that practically all of these methods should be deleted and intends so to recommend next year. An expression of opinion from members of the association on this point will be appreciated<sup>1</sup>.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 88.

No report on the separation of meat proteins was given by the associate referee.

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No report on the determination of sugar in meat products was given as no associate referee was appointed.

## REPORT ON GELATIN.

By E. H. BERRY (U. S. Food and Drug Inspection Station, Chicago, Ill.),  
*Referee.*

The report made in 1923<sup>1</sup> showed rather conclusively that the tentative and alternative methods for the determination of copper and zinc in gelatin<sup>2</sup> gave very unsatisfactory results. In fact, the results obtained in 1923 confirmed the results of the work performed in 1922.

Accordingly, the referee was instructed to continue the study of methods for the determination of these metals. He regrets that the amount of work performed during 1924 hardly warranted making a report. Again this year sufficient time was not available to accomplish as much as had been planned. However, it is felt that enough has been done to warrant recommendations for collaborative work for next year.

It will, no doubt, be recalled that both the tentative and alternative methods, as well as other methods in use by various analysts, direct that the gelatin be hydrolized with acid. Considerable time was required to work out details using this method of treating the gelatin. However, the results obtained were quite unsatisfactory as a whole in spite of the fact that many variations were tried.

A method for the determination of copper and zinc in gelatin was published by Raymond Hertwig<sup>3</sup>. Hertwig ashed 20 grams of gelatin and determined the metals in the ash. The smallness of the sample used is the chief objection to this procedure. Another method similar to the Hertwig method, published by Roger M. Mehurin<sup>4</sup>, also requires ashing of the gelatin. Mehurin directs that from 20-40 grams be ashed in a muffle and states that the sample must not be placed in the muffle until the latter has reached the proper temperature or the material will boil over. Observing this precaution, it was found that 50 grams of gelatin can be ashed in a porcelain dish of 150 cc. capacity. Using these two methods as a basis, the following details have been worked out:

### METHOD.

Ash 50 grams of gelatin in a platinum or porcelain dish of about 150 cc. capacity. Regulate the temperature so that the muffle presents a barely visible red when the

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<sup>1</sup> *This Journal*, 1923, 7: 135.

<sup>2</sup> *Methods of Analysis*, A. O. A. C., 1925, 256.

<sup>3</sup> *This Journal*, 1923, 7: 41.

<sup>4</sup> *J. Ind. Eng. Chem.*, 1923, 15: 942.

material is placed in it and do not increase it at any time. If necessary, hasten the ashing by leaching the well charred mass with water. Moisten the ash with water, add approximately 5 cc. of concentrated hydrochloric acid, and evaporate to dryness. Dissolve in 10 cc. of 1 + 1 hydrochloric acid, add 40 cc. of water, and filter. Neutralize the filtrate with ammonium hydroxide, using methyl orange, and add sufficient excess to precipitate all iron and aluminium and assure solution of the copper and zinc. Heat the solution to about 80°C. Filter and wash the precipitate with a 3 per cent solution of ammonium chloride containing about the same quantity of free ammonium hydroxide as the original solution. Dissolve the precipitate with dilute hydrochloric acid, and reprecipitate and filter as before. Neutralize the combined filtrates with hydrochloric acid, adding a slight excess of acid. Pass hydrogen sulfide into the hot solution until it is cold. Filter out the copper sulfide, observing the proper precautions to prevent its oxidation and wash with 3 per cent ammonium chloride solution saturated with hydrogen sulfide. Dissolve the copper sulfide on the filter paper by washing with hot nitric acid (1 + 3) and wash with hot water. Evaporate the solution to dryness. Add a small quantity of water and make alkaline with ammonium hydroxide. Heat on the steam bath away from hydrogen sulfide fumes until all ammonia is expelled, adding water from time to time. Do not evaporate to dryness. Filter the neutral solution into a 50 cc. graduated flask, add 5 cc. of ammonium nitrate (10 grams per 100 cc.) and five drops of potassium ferrocyanide solution (4 grams per 100 cc.), make to mark, and mix. Prepare a standard containing approximately the same quantity of copper as the solution prepared from the sample. Match the two solutions, using a colorimeter. (Should a colorimeter not be available, standards containing varying quantities of copper may be prepared and the comparison made in Nessler tubes.) From these comparisons calculate the quantity of copper in the sample.

Neutralize the filtrate containing the zinc with ammonium hydroxide and make slightly acid with hydrochloric acid. Add 10–15 cc. of 50 per cent ammonium acetate solution. Heat nearly to boiling and pass hydrogen sulfide into the solution until the solution is cold. Allow the precipitate to settle and filter on a paper or Gooch crucible. Wash the precipitate with 2 per cent ammonium nitrate solution saturated with hydrogen sulfide, ignite to constant weight, and weigh as zinc oxide.

Very concordant results were obtained by the referee using this proposed method. The copper determinations ranged from 5–8 parts per million and seven determinations of zinc ranged from 82–104 with an average of 92 parts per million on a gelatin known to contain approximately 100 parts of zinc.

#### RECOMMENDATION<sup>1</sup>.

It is recommended that the study of methods for the determination of copper and zinc be continued. This study should include collaborative work on the method described in this report and on the tentative methods of the association.

*C. A. Browne:* This association, as probably most of the members know, sprang into existence as a foster child of the United States Department of Agriculture. It held its first meetings in the main building of the Department, and for many years its annual proceedings and methods of analysis were published as bulletins by the Department. Al-

<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 88.

though the association left the shelter of its old home many years ago, it has continued the happy custom of welcoming at its annual meetings each year either the secretary, or the assistant secretary, of the Department, and of hearing from him a few words of greeting. Forty years ago it was that Secretary Coleman gave the first address. Following that time, Secretaries Wilson, Meredith, Wallace, and others have addressed our association.

We are very fortunate in having with us today a man whom you all know, the Hon. R. W. Dunlap. Mr. Dunlap will now address us.

*Mr. Dunlap:* MR. CHAIRMAN, LADIES AND GENTLEMEN: Secretary Jardine regrets his inability to greet you this afternoon. I am here as his substitute, and you know the substitute is never equal to the principal. However, it is necessary to have some one in reserve, whether it is a football team or any other organization. So, as I happen to be the Assistant Secretary, the Secretary asked me if I would not come over here and read the short address which he has prepared.

#### ADDRESS BY THE SECRETARY OF AGRICULTURE— THE HONORABLE W. M. JARDINE.

##### DIVERSIFYING THE USES OF FARM CROPS.

It is a pleasure to extend the greetings of the United States Department of Agriculture to any group of scientists. No one realizes more thoroughly than I the necessity for sound, accurate research in every branch of science as a basis for agricultural progress. I am especially happy to welcome your association—the Association of Official Agricultural Chemists—because of the immediate relationship of your work to agriculture and because of the achievements that you have made in developing accurate methods of analysis. These methods are valuable in promoting chemical research, as well as in making human foods, feeds for livestock, and other products most dependable. Our own Bureau of Chemistry here in the Department has for years recognized your association as one of the most significant with which to cooperate in the advancement of economic chemistry.

The immediate object of your association is, of course, to develop methods of analysis, but the accomplishments that you have made show that you recognize these methods as merely means to an end—the service of agriculture and of the industries that utilize agricultural products.

Perhaps the greatest possibility for service to agriculture on the part of the chemist in the immediate future lies in the more diversified utilization of crops. For a number of years authorities in agriculture have pointed out the importance of diversified production, and farmers are more and more adopting plans looking in this direction. We need, however, diversity of utilization as well as diversity of production. From an

economic standpoint, flexibility of methods for utilizing a plant crop is highly desirable. For instance, owing to the relative price of sugar and sirup during the last season, a larger return could be obtained by the growers of cane in Louisiana by converting a greater proportion of their crop into sirup. In some other seasons the reverse has been true, it having been found more profitable to make cane sugar than to make cane sirup. Chemical research is assisting the manufacturers in Louisiana in developing methods for the production on a larger scale of a grade of cane sirup that will meet the demands of a wider market. The cane growers of Louisiana will hereafter have two markets instead of one.

Again, chemists have developed processes for the utilization of cull oranges and lemons that enable the grower to find a market for fruit that was formerly wasted. Several products are now manufactured from corn, but only a beginning has been made. Similar possibilities exist in the case of many other important crops. The development of varied methods of utilizing crops will be beneficial to everybody concerned—producer, distributor, and consumer.

It is an interesting and significant indication of the interrelation of all science and industry in the world today that chemistry in this way is proving an important part of a sound, economic policy in connection with agriculture. Agriculture is no single science. In the variety and complexity of its interests it embraces many sciences, all related one to another, and all alike important to rural progress. Chemistry is one of the most fundamental of these sciences, and I congratulate your association on the way in which it has directly applied this basic science to the solution of pressing agricultural problems.

No report on spices and other condiments was given by the referee.

## REPORT ON CACAO PRODUCTS.

By E. M. BAILEY (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Referee*.

In the report of the referee last year<sup>1</sup> reference was made to collaborative studies of the official and tentative methods for the analysis of cacao products as applied to three types of such products prepared according to known formulas under conditions prevailing in commercial practice. The experimental material was supplied by courtesy of the Bureau of Chemistry, representatives of which supervised the manufacture of the several products herein discussed. The ingredients were analyzed by E. R. Miller of the New York Food and Drug Inspection Station, and to the list of collaborators whose cooperation was acknowl-

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<sup>1</sup> *This Journal*, 1925, 8: 701.

edged last year should be added C. E. Shepard of the Connecticut Experiment Station, New Haven.

# FORMULAS AND ANALYSES OF INGREDIENTS OF COLLABORATIVE CHOCOLATE PRODUCTS.

The basic data on the experimental materials are as follows:

**SAMPLE D.**—Bitter liquor made from 280 pounds of shell-free nibs and 11.87 pounds of shell. The nibs contained 3.22 per cent of moisture and 54.29 per cent of fat. The shells contained 8.28 per cent of moisture and 4.53 per cent of fat.

*Analysis of bitter liquor:* Moisture, calculated, 3.43 per cent, found 2.13 per cent; fat, calculated, 52.27 per cent, found 52.17 per cent.

**SAMPLE 6 C. S.**—Sweet chocolate made from 21.34 pounds of Liquor C, 141.75 pounds of sugar, and 77.5 pounds of cacao butter. Liquor C was made from 280 pounds of shell-free nibs and 8.71 pounds of shell.

*Analysis of Liquor C:* Moisture, calculated, 3.37 per cent, found 1.82 per cent; fat, calculated, 52.78 per cent, found 53.23 per cent.

*Analysis of sugar (Conf. XX):* Moisture 0.03 per cent, sucrose 99.97 per cent.

*Analysis of cacao butter:* Moisture, 0.05 per cent; fat, by difference, 99.95 per cent.

*Analysis of Sample 6 C. S., calculated:* Mix, 240.59 pounds, contained moisture 0.47 pounds = 0.20 per cent; fat 88.82 pounds = 36.91 per cent; sucrose 141.71 pounds = 58.90 per cent.

**SAMPLE 8 D. M.**—Sweet milk chocolate made from 17.5 pounds of Liquor D, 113 pounds of sugar, 75.5 pounds of cacao butter, 30.5 pounds of Cremora A, and 3.5 pounds of milk fat.

*Analysis of Liquor D:* As given for Sample D.

*Analysis of sugar:* As given for Sample 6 C. S.

*Analysis of cacao butter:* As given for Sample 6 C. S.

*Analysis of milk fat:* Moisture, 0.07 per cent; fat, by difference, 99.93 per cent.

*Analysis of Cremora A:* Moisture, 4.57 per cent; ash, 6.05 per cent; water-soluble ash, 2.27 per cent; water-insoluble ash, 3.78 per cent; alkalinity of water-soluble ash, 2.5 cc. normal sodium hydroxide per 100 grams; alkalinity of water-insoluble ash, 50.5 cc. normal sodium hydroxide per 100 grams; lactose, 37.07 per cent; casein, 17.25 per cent; fat, 26.37 per cent.

*Analysis of Sample 8 D. M., calculated:* Mix, 240 pounds, contained moisture, 1.94 pounds = 0.78 per cent; fat, 96.13 pounds = 40.05 per cent; sucrose, 112.97 pounds = 47.07 per cent; lactose, 11.65 pounds = 4.85 per cent; casein, 5.26 pounds = 2.19 per cent; milk fat, 11.54 pounds = 4.81 per cent.

## METHODS.

The following directions as to methods were sent to all collaborators. The sections and paragraphs cited are those given in the first edition (1920) of *Methods of Analysis*, A. O. A. C., but changes to be made in the revision were anticipated so that the directions as given agree with those in effect at the present time<sup>1</sup>.

1. Prepare the samples for analysis as directed under XXIV, 1.

2. *Moisture.*—(a) Follow present official method as directed under VII, 2 (not VIII, 2).

In reporting results state whether dried in hydrogen or in vacuum.

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925.

(b) Determine moisture also by drying in an air oven at 105°C.

3. *Ash*.—Follow present official method as directed under VII, 4 (not VIII, 4).

4. *Ash insoluble in acid*.—Proceed as directed under XXIV, 4.

5. *Soluble and insoluble ash*.—Proceed as directed under XXIV, 5.

6. *Alkalinity of soluble ash*.—Proceed as directed under XXIV, 6.

7. *Alkalinity of insoluble ash*.—Proceed as directed under XXIV, 7.

8. *Crude fiber*.—Proceed as directed under XXIV, 9. This section refers to the crude fiber method as outlined in VII, 66, which was dropped from the official methods about two years ago. The new crude fiber method as adopted at the last A. O. A. C. meeting<sup>1</sup> should be substituted here in place of VII, 66. A copy of the new procedure is enclosed for your guidance. The other provisions as given in XXIV, 9, still hold as applied to cacao products.

9. *Fat*.—Proceed as directed under XXIV, 12.

10. *Milk fat*.—Separate the fat for the determination of the Reichert-Meissl number as directed under XXIV, 13, which refers to XXIV, 15. The bulk of the solvent may be removed by distillation and the last traces, by evaporation at a temperature not exceeding 100°C. Estimate the milk fat as directed under XXIV, 14.

11. *Sucrose and lactose*.—Proceed as directed under XXIV, 15.

12. *Casein*.—Proceed as directed under XXIV, 16.

## RESULTS OF ANALYSES.

The results submitted by collaborators are compiled in Table 1. The calculated values appearing in the table are inserted as a guide and are not to be understood as exact theoretical values; it will be noted, however, that in most cases they are quite close to the average of collaborative results. A copy of the complete compilation was submitted to each collaborator so that individual results might be carefully scrutinized; in this way the inclusion of figures which would be unfair to the analysts and to the methods as well was practically avoided. It is believed, therefore, that this compilation represents a very fair and thorough test of the association's methods as applied to the types of products examined.

## COMMENTS OF COLLABORATORS.

*J. Callaway:* Our interpretation of the directions for crude fiber was that both acid and alkali filtrations should be made on paper, and this was done. There is a small source of error in the method, as outlined, due to the difference in weight between asbestos after drying at 110°C. and after ignition. This difference in weight has been noted by us on previously ignited asbestos and amounts to as much as 3 mg. per gram on the asbestos used in this laboratory. The method for crude fiber did not direct that any correction be made for this difference and none has been made in the results reported.

The tentative method for casein seems to need some further standardization, as the present directions allow too much leeway in the time of boiling, etc.

No other comments in the way of criticism of methods were received. In the light of results submitted for casein, Callaway's remark is of interest.

<sup>1</sup> *This Journal*, 1926, 9: 30.



## DISCUSSION OF RESULTS.

## CRUDE FIBER.

The results for crude fiber may be summarized as follows:

	D. <i>per cent</i>	6 C. S. <i>per cent</i>	8 D. M. <i>per cent</i>
Maximum.....	3.67	0.81	0.75
Minimum.....	3.06	0.23	0.26
Average.....	3.36	0.50	0.46
Variation.....	0.61	0.58	0.49

The variation between extreme results is roughly 0.5 per cent regardless of the amount of fiber present. If the extreme high and low results are excluded in all cases, the variation is less. Thus in Sample D, nine results are within a range of 0.42 per cent, and in Sample 8 D. M. eight results are within a range of 0.23 per cent. If all results are considered, the limit of error in the two last named samples is greater in magnitude than that of the average amount of fiber present, and it is from 50-80 per cent of the total amount when based on selected results.

## FAT.

The summarized data for fat are as follows:

	D. <i>per cent</i>	6 C. S. <i>per cent</i>	8 D. M. <i>per cent</i>
Maximum.....	53.07	37.30	40.71
Minimum.....	51.83	35.98	39.66
Average.....	52.31	36.89	40.17
Variation.....	1.24	1.32	1.05
Calculated.....	52.27	36.91	40.05

Results vary from 1-1.3 per cent for the three samples. In the case of Sample D, 80 per cent of the results are within a range of 0.66 per cent; in Samples 6 C. S. and 8 D. M. like proportions are within 0.31 and 0.72 per cent, respectively.

## CASEIN.

The following summary of casein results may be made:

	D. <i>per cent</i>	6 C. S. <i>per cent</i>	8 D. M. <i>per cent</i>
Maximum.....	1.66	0.37	2.22
Minimum.....	0.95	0.10	1.87
Average.....	1.22	0.19	2.06
Variation.....	0.71	0.17	0.35
Calculated.....	none	none	2.19

A number of collaborators obtained values for "casein" in two of the samples that contained no milk constituents. Whether those analysts reporting "none" actually obtained blanks, or traces which were ignored, cannot be stated. However, it is evident that the method as outlined at present yields nitrogenous material which is reported as casein where

no casein is present. A comparison of results for casein on Samples D and 6 C. S. suggests that cacao protein is the disturbing factor since the amount of chocolate liquor in Sample 6 C. S. is about one-tenth of that in Sample D, and the amount of "casein" reported in Sample 6 C. S. is decreased in about that proportion. It is recognized, however, that 0.1 per cent of casein represents less than 0.02 per cent of nitrogen, which is a magnitude beyond the limit of accuracy for determining nitrogen by the method employed. In Sample 8 D. M. the amount of cacao material is even less than in Sample 6 C. S. so that the error due to cacao nitrogen reckoned as "casein" in both these samples is probably negligible.

#### SUCROSE AND LACTOSE.

The summary for sucrose and lactose is as follows:

	6 C. S. SUCROSE per cent	8 D. M. SUCROSE per cent	LACTOSE per cent
Maximum.....	59.54	47.10	6.30
Minimum.....	56.59	44.64	2.95
Average.....	58.09	46.35	4.65
Variation.....	2.55	2.46	3.35
Calculated.....	58.90	47.07	4.71

It appears that an experimental error of about 2.50 per cent may be expected among experienced analysts in determining sucrose in amounts of the magnitude shown in these two products. Excluding three results in the case of Sample 6 C. S. and one in the case of Sample 8 D. M., the remaining results fall within ranges of variation of 2 per cent and 1.6 per cent, respectively.

The limit of error in determining lactose is 3.35 per cent if all results are considered and 1.7 per cent based on the seven figures in closest agreement. The range of variation is about 35 per cent of the total amount present when based on seven selected results, and more than twice that when all results are considered. This is disregarding results showing lactose in 6 C. S., two of which can hardly be called negligible.

#### MILK FAT IN SAMPLE 8 D. M.

This summary is as follows:

	per cent
Maximum.....	5.28
Minimum.....	3.55
Average.....	4.33
Variation.....	1.73
Calculated.....	4.81

Excluding the extremes the range of variation is 1.27 per cent, but on the basis of all figures reported the variation is about 36 per cent of the total amount of milk fat present.

In Samples D and 6 C. S. results for milk fat were uniformly negative with one exception which is unexplained.

#### MILK SOLIDS.

The milk solids calculated from reported determinations are fairly close to the figure obtained entirely from calculated values. In one or two cases, however, a compensation of plus and minus variations takes place to effect this agreement.

#### COMPARISON OF PRESENT AND FORMER RESULTS.

In 1920 Bloomberg<sup>1</sup> reported a critical study of methods for the determinations of fat, sucrose, lactose, and casein in milk chocolate, and the procedures recommended at that time for these determinations are the present official and tentative methods. It is of interest to compare the results obtained then with those reported here.

##### *Summary of data on analyses of milk chocolate.*

	FAT per cent	SUCROSE per cent	LACTOSE per cent	CASEIN per cent
Referee's Sample 1920				
Calculated.....	....	40.00	7.00	4.14
Maximum.....	33.07	43.81	8.19	4.16
Minimum.....	31.56	39.33	6.41	3.30
Average.....	32.77	40.50	7.06	3.72
Range of variation.....	1.51	4.48	1.78	0.86
Referee's Sample 1925				
Calculated.....	40.05	47.07	4.71	2.19
Maximum.....	40.71	47.10	6.30	2.22
Minimum.....	39.66	44.64	2.95	1.87
Average.....	40.16	46.35	4.64	2.05
Range of variation.....	1.05	2.46	3.35	0.35

The combined data represent the results of about twenty collaborators. So far as agreement is concerned, the results are not all that could be desired, although averages are close to the calculated values. Most of the analysts taking part in the earlier work, moreover, reported that the fat was more or less contaminated with cacao alkaloids, but the extent of the error thus introduced was not determined. Recently Lepper and Waterman<sup>2</sup> have suggested a modification which obviates this difficulty.

As compared with the new results reported this year the earlier figures show a wider range of variation between the extremes for sucrose, but the results for lactose are in closer agreement. It has been pointed out in criticism of the method for determining these two sugars that slight variations in polariscopic readings considerably influence the final results. The recent results for casein are rather more favorable to the method

<sup>1</sup>*This Journal*, 1920, 3: 490.

<sup>2</sup>*Ibid.*, 1925, 8: 705.

than those previously reported by collaborators, although the preliminary work<sup>1</sup> that was done by the author of the method when it was under consideration for adoption was very satisfactory, at least for amounts of casein from 1-4.5 per cent.

As applied to chocolate liquor the present methods are fairly satisfactory, as shown, for example, by a comparison of values for total ash and for crude fiber on the basis of the moisture- and fat-free chocolate. Thus, on this basis, the values for ash range from 7-7.9 per cent, while seven out of the ten results reported show a variation of only 0.4 per cent. In the case of crude fiber, where a greater range is to be expected, the variation between extreme values is 1.4 per cent, but eight of the ten results vary by only 0.7 per cent. In products of the types represented by Samples 6 C. S. and 8 D. M., however, in which moisture- and fat-free chocolate constitutes only about 5 per cent of the whole, analytical variations become magnified to such a degree that it is difficult or impossible to arrive at accurate conclusions with respect to the character of the cacao material present.

To what extent the difficulties cited can be overcome by improvement in methods remains to be determined. Within the last two years two modifications of the method for fat determination have been suggested, and they have both been studied collaboratively during the past year. The method for casein should be studied with particular reference to its behavior when applied to chocolate products that are unmixed with sugar, or milk, or both. As previously noted, in the case of sweet chocolate and sweet milk chocolate the error introduced by the possible evaluation of cacao nitrogen as casein does not appear to be of much concern because of the greatly reduced proportion of cacao constituent. The method for the determination of sucrose and lactose suggests itself for further study. Probably the results for moisture by the vacuum process would have been improved if the directions to collaborators had specified the degree of vacuum.

This concludes the report of work done according to the program of 1923-24.

#### WORK OF 1924-25.

The recommendations for the past year were (1) the continued study of methods for the estimation of shell in cacao products; (2) the continued study of methods for the detection of foreign fats in cacao products containing milk constituents; (3) the study of the crude fiber content of alkalized cacao products; and (4) the study of two methods recently proposed for the determination of fat.

Upon the first project the associate referee reports that progress has been made, but the conclusions are tentative only and do not warrant a formal report at this time.

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<sup>1</sup> *This Journal*, 1920, 3: 488.

Upon the second project the associate referee reports that considerable study has been devoted to the test for detecting cocoanut and palm kernel oils in cacao butter and the fat from milk chocolate with the view to correcting the defects of the test as revealed by collaborative study last year. The test has not yet been perfected.

Owing to pressure of other work the associate referee assigned to study the fiber content of alkaliized products has not been able to give attention to this subject.

The study of methods for the determination of fat in cacao products has included trials of the methods cited in the recommendations in comparison with the present official method and a modification of that method suggested by the procedure of Lepper and Waterman. These authors have proposed the use of petroleum ether as a solvent in place of ethyl ether, thus eliminating the error due to the extraction of cacao alkaloids by ethyl ether. Their method further avoids the necessity for preliminary drying of the sample, and other details of technique greatly reduce the time required to secure accurate results.

The advantages of petroleum ether as a solvent in this determination cannot be denied, but the possibility of modifying the present official method by simply changing from ethyl ether to petroleum ether was so obvious that the referee was led to suggest this revision to collaborators. With the substitution of petroleum ether as the solvent, advantage is automatically taken of the fact that preliminary drying is unnecessary. While the use of the Knorr extraction tube may considerably shorten the actual extraction period, a continuous extraction with an apparatus of the Johnson type, for example, is not necessarily a disadvantage in this respect because no attention is necessary and other work can be carried on in the meantime.

Samples were submitted to five collaborators, all of whom reported. The experimental samples were the same products that were used in the studies conducted a year ago and were furnished by courtesy of the New York Food and Drug Inspection Station.

The following directions were sent to collaborators:

Determine fat in each of the three samples by each of the following methods:

1. A. O. A. C. Official Method.
2. Feldstein Method, Jour. A. O. A. C. VIII, 1, p. 75.
3. Lepper-Waterman Method (as reported to the A. O. A. C., 1924, method enclosed).
4. The Official Method (A. O. A. C.), using 2 grams of material *without drying*, and using petroleum ether (redistilled below 60°C. as in the Lepper-Waterman method.) Note the percentage of fat found at the end of the 4 hour extraction, also the increase after grinding and reextracting as directed in the method.

NOTE: *Methods 1 and 4:* The material should be stratified with asbestos in the extraction tube to prevent clogging. It is advisable in both cases to allow the solvent to run through completely in the cold; then heat may be applied for the continuous extraction.

*Method 2:* Note color of the extracted fat and its behavior on drying.

The chemists reporting, and to whom acknowledgment is made, are M. L. Offutt, New York Station; H. W. Haynes, Boston Station; C. E. Shepard and W. T. Mathis, Connecticut Agricultural Station, New Haven; and R. L. Horst, New Orleans Station.

The results obtained by the several analysts are summarized in Table 2.

TABLE 2.  
*Comparative determinations of fat in cacao products.*

COLLABORATOR	I OFFICIAL METHOD	II FELDSTEIN METHOD	III LEPPER- WATERMAN METHOD	IV OFFICIAL METHOD, FIRST EXTRACTION	MODIFIED SECOND EXTRACTION	ROESE- GOTTLIEB METHOD
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
SAMPLE D.						
M. L. Offutt	52.28	52.34	52.00	52.01	52.05	...
C. E. Shepard	53.07	51.95	52.03	51.77	51.86	51.87
W. T. Mathis	52.63	52.18	51.67	.....	51.81	...
H. W. Haynes	51.94	51.39	.....	51.34	51.55	....
R. L. Horst	51.73	49.15	51.54	.. ..	51.41	....
Maximum	53.07	52.34	52.03	52.01	52.05	.....
Minimum	51.73	49.15	51.54	51.34	51.41	.....
Average	52.33	51.40	51.81	51.71	51.74	51.87
Variation	1.34	3.19	0.49	0.67	0.64	.....
SAMPLE 6 C. S.						
M. L. Offutt	37.01	37.24	36.86	36.59	36.63	.....
C. E. Shepard	37.30	38.02	36.79	36.65	36.76	36.79
W. T. Mathis	37.39	37.77	36.74	.....	36.71	.....
H. W. Haynes	37.07	37.08	.....	36.67	36.78	.....
R. L. Horst	36.95	37.36	36.82	.....	36.55	.....
Maximum	37.39	38.02	36.86	36.67	36.78	.....
Minimum	36.95	37.08	36.74	36.59	36.55	.....
Average	37.14	37.49	36.80	36.64	36.69	36.79
Variation	0.44	0.94	0.12	0.08	0.23	...
SAMPLE 8 D. M.						
M. L. Offutt	40.18	40.71	40.08	39.46	39.59	....
C. E. Shepard	40.71	41.69	40.08	40.01	40.16	40.26
W. T. Mathis	40.85	41.03	40.14	.....	40.06	.....
H. W. Haynes	40.26	39.21	.....	40.22	40.20	.. ..
R. L. Horst	40.22	38.99	39.79	.....	40.81	....
Maximum	40.85	41.69	40.14	40.22	40.81	...
Minimum	40.18	38.99	39.79	39.46	39.59	.....
Average	40.44	40.33	40.02	39.90	40.16	40.26
Variation	0.67	2.70	0.35	0.76	1.22	.....

TABLE  
Collaborative analyses

COLLABORATOR	MOISTURE		ASH					
	In vacuum at 100°C.	In air at 105°C.	Total	Insoluble in acid	Soluble in water	Insoluble in water	Alkalinity of soluble, cc. N acid per 100 grams of sample	Alkalinity of insoluble, cc. N acid per 100 grams of sample
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Calculated	3.83	....	....	....	....	....	....	....
D. H. McIntire	2.53	2.38	3.38	0.06	1.62	1.76	11.3	26.4
J. Callaway, Jr.	2.96	2.76	3.35	0.06	1.62	1.73	15.0	25.0
S. C. Rowe	2.56	3.42	3.42	0.05	1.47	1.95	11.0	12.0
M. L. Offutt	2.55	2.58*	3.58	0.08	1.85	1.73	13.0	23.0
C. A. Greenleaf	2.61†	2.61	3.24	0.08	1.44	1.80	11.2	27.0
J. T. Field	2.20†	2.01	3.24	0.32	1.60	1.64	13.0	24.0
R. L. Horst	2.63§	2.33	3.40	0.04	1.70	1.70	14.3	23.0
Ferris and Stoner	2.30	2.32	3.46	0.10	1.61	1.85	13.4	30.3
C. E. Goodrich	3.06**	....	3.25	0.09	1.88	1.37	12.1	14.8
H. W. Haynes	1.88	1.90	3.20	....	1.39	1.81	11.7	24.3
C. E. Shepard	2.87	2.46	3.19	0.14	1.31	1.89	12.3	25.4
Maximum	3.06	3.42	3.58	0.32	1.88	1.95	15.0	30.3
Minimum	1.88	1.90	3.19	0.04	1.31	1.37	11.0	12.0
Average	2.55	2.47	3.33	0.10	1.59	1.74	12.6	23.2
Calculated	0.20	....	....	....	....	....	....	....
D. H. McIntire	0.30	0.25	0.34	0.08	0.16	0.18	1.6	2.4
J. Callaway, Jr.	0.49	0.47	0.35	0.02	0.18	0.17	1.8	2.3
S. C. Rowe	0.33	0.39	0.37	None	0.20	0.17	1.6	2.9
M. L. Offutt	0.20	0.28*	0.34	None	0.25	0.09	3.0	2.0
C. A. Greenleaf	0.41†	0.40	0.31	None	0.15	0.16	1.4	2.7
J. T. Field	0.25†	0.22	0.35	0.02	0.16	0.19	2.0	4.5
R. L. Horst	0.29§	0.28	0.33	None	0.17	0.12	3.0	3.0
Ferris and Stoner	0.25	0.15	0.36	0.01	0.10	0.26	1.8	4.2
C. E. Goodrich	0.26**	....	0.35	None	0.20	0.15	1.7	1.9
H. W. Haynes	0.17	0.21	0.36	....	0.14	0.22	1.7	5.0
C. E. Shepard	0.32	0.33	0.29	0.06	0.10	0.19	1.0	2.9
Maximum	0.49	0.47	0.37	0.08	0.25	0.26	3.0	5.0
Minimum	0.17	0.15	0.29	0.02	0.10	0.09	1.0	1.9
Average	0.29	0.29	0.34	0.03	0.16	0.17	1.9	3.1
Calculated	0.78	....	....	....	....	....	....	....
D. H. McIntire	0.65	0.55	0.97	0.04	0.35	0.62	3.7	9.4
J. Callaway, Jr.	1.14	1.13	0.97	0.01	0.28	0.69	1.5	8.6
S. C. Rowe	0.60	0.81	0.99	None	0.32	0.67	2.3	7.0
M. L. Offutt	0.68	0.50*	0.94	None	0.40	0.54	3.0	8.5
C. A. Greenleaf	0.97†	1.01	0.97	0.01	0.27	0.70	1.4	9.2
J. T. Field	0.50†	0.46	0.98	0.07	0.26	0.72	1.5	11.5
R. L. Horst	0.64§	0.71	0.96	None	0.26	0.70	2.0	10.0
Ferris and Stoner	0.89	0.77	0.89	0.04	0.30	0.59	5.1	12.0
C. E. Goodrich	0.86**	....	0.98	None	0.29	0.69	1.0	9.6
H. W. Haynes	0.40	0.48	0.98	....	0.31	0.67	3.3	11.0
C. E. Shepard	0.75	1.00	0.98	0.06	0.32	0.66	2.8	8.3
Maximum	1.14	1.13	0.98	0.07	0.40	0.72	5.1	12.0
Minimum	0.40	0.46	0.89	0.01	0.26	0.54	1.0	8.3
Average	0.68	0.74	0.96	0.03	0.30	0.65	2.5	9.3

\* At 110°C.

† Vacuum 29 inches, 98°C.

‡ Johnson extractor apparatus used.

§ Vacuum 25 inches, 97°-99°C.

\*\* Method not stated.

1.

*of cacao products.*

CRUDE FIBER	SUCROSE	LACTOSE	FAT	REICHERT- MEISSL NO. OF FAT	MILK FAT	CASEIN	MILK SOLIDS
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
<b>D.</b>							
....	....	....	52.27	....	....	....	....
3.06	None	None	52.17	0.29	None	None	....
3.67	1.03	None	52.04	0.35	None	1.05	....
3.28	None	None	52.01	....	None	Trace	....
3.21	....	....	52.24	....	....	....	....
3.56	....	....	51.83†	0.20	None	1.10	....
3.30	None	None	52.56	..	None	1.07	....
3.55	None	None	....	..	None	None	....
3.53	0.40	None	52.46	....	None	1.66	....
3.29	....	....	52.67	....	....	....	....
3.29	....	....	52.06	....	None	0.95	....
3.24	0.08	None	53.07	0.48	None	1.31	....
3.67	1.03	....	53.07	0.48	....	1.66	....
3.06	0.40	....	51.83	0.20	....	0.95	....
3.36	0.50	....	52.31	0.33	....	1.19	....
<b>6 C. S.</b>							
....	58.90	....	36.91	...	....	..	....
0.31	56.59	None	37.04	0.35	None	None	....
0.45	58.05	0.88	37.08	0.38	None	None	....
0.23	59.28	None	36.91	..	None	None	....
0.30	57.82	....	37.05	....	....	....	....
0.62	....	....	36.18†	0.20	None	0.37	....
0.51	59.54	None	35.98	....	2.31	None	....
0.81	57.01	....	....	....	None	None	....
0.72	57.60	0.35	37.28	....	None	0.10	....
0.52	58.91	None	36.99	....	....	....	....
0.42	57.49	1.68	37.04	....	None	None	....
0.59	58.65	None	37.30	0.50	None	0.10	....
0.81	59.54	1.68	37.30	0.50	...	0.37	....
0.23	56.59	0.35	35.98	0.20	....	0.10	....
0.49	58.09	0.97	36.88	0.35	...	0.19	....
<b>8 D. M.</b>							
....	47.07	4.71	40.05	....	4.81	2.19	12.9
0.26	44.64	3.38	40.17	3.35	4.87	1.99	11.3
0.53	46.75	4.61	40.24	3.09	4.40	2.16	12.3
0.34	47.09	2.95	40.20	....	4.11	2.22	10.3
0.30	45.83	4.00	40.51	3.27	4.77	2.22	12.1
0.31	....	....	39.79†	2.60	3.55	2.10	....
0.36	45.86	5.25	39.66	....	4.55	None	....
0.73	46.94	5.34	....	....	....	2.01	....
0.75	45.50	5.70	40.45	....	3.60	1.87	12.2
0.48	46.98	4.46	40.06	....	....	....	....
0.44	46.85	6.30	39.86	....	3.87	2.00	13.3
0.52	47.10	4.49	40.71	3.55	5.28	1.95	12.8
0.75	47.10	6.30	40.71	3.55	5.28	2.22	13.3
0.26	44.64	2.95	39.66	2.60	3.55	1.87	10.3
0.45	46.35	4.64	40.16	3.17	4.33	2.05	12.0



## COMMENTS OF COLLABORATORS.

Collaborators have commented as follows:

*M. L. Offutt:* Continuous extraction method would seem to be more efficient where a number of samples are to be run at one time.

*C. E. Shepard:* Fat by official method high by reason of extraction of alkaloid. Fat by Feldstein method discolored in case of Samples 6 C. S. and 8 D. M. and vapors other than those of the solvent noted on drying the fat. In the Roese-Gottlieb method emulsions may form; they may be broken up, however, by the addition of 0.5–1.0 gram of sodium chloride. Results are in close agreement with modified official method. In the latter, however, the 4 hour period was not adhered to but extraction allowed to continue overnight. By the Lepper-Waterman method fat residues were normal in appearance and results were in good agreement with the modified method.

*H. W. Haynes:* Fats extracted by Method II were slightly discolored and in some cases badly contaminated with charred material.

*R. L. Horst:* In Method II, for Samples 6 C. S. and 8 D. M. the extracted fat was of a yellow-brown color and for D colorless. Upon drying the two first mentioned became dark brown in color.

## DISCUSSION OF RESULTS.

Since the object of this study is to secure a method that will give correct and closely agreeing results in the hands of different analysts the results reported should be judged on the basis of the variations shown between the extreme figures reported in each group. On this basis the following summary may be made:

METHOD	VARIATION RANGE per cent	NO. OF RESULTS REPORTED
I. Official method.....	0.4–1.3	15
II. Feldstein method.....	0.9–3.2	15
III. Lepper-Waterman method....	0.1–0.5	12
IV. Official method, modified.....	0.2–1.2	15

It appears that Method III gave results within a narrower range of variation than any of the other methods studied. It should be noted, however, that the full number of results was not reported in this case, and further, that if a single result by Method IV be excluded the variation range for that method becomes 0.2–0.6 per cent. It is of interest to note that where comparisons with the Roese-Gottlieb method can be made, the results by Methods III and IV are in close agreement with those obtained by that procedure.

Because of the obvious advantage in the use of petroleum ether as a solvent and the close agreement in the results obtained by different analysts, the recommendation of the Lepper-Waterman method as an official method for the determination of fat in cacao products seems justified. The modified official method appears to possess possible advantages also and to deserve further consideration. Its recommendation as a separate method, or a change in the description of the Lepper-Waterman method to include specified forms of continuous extraction, may later be warranted.

RECOMMENDATIONS<sup>1</sup>.

It is recommended—

(1) That the Lepper-Waterman method (as reported in 1924) be adopted as an official method for the determination of fat in cacao products (first reading).

(2) That the modified method described in this report be further studied.

(3) That the recommendations of last year with reference to the estimation of shell, to the detection of foreign fats in milk chocolate, and to the crude fiber content of alkalinized cacao products, be repeated.

(4) That the possibility of further improving the methods for determining casein and the distribution of sugars should be considered.

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No report on microscopical methods for cacao products was given by the associate referee.

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No report on crude fiber in cacao products was given by the associate referee.

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No report on cacao butter was given by the associate referee.

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No report on naval stores was given by the referee.

## REPORT ON TURPENTINE.

By V. E. GROTLISCH (Bureau of Chemistry, Washington, D. C.), *Associate Referee*.

The work on turpentine this year was devoted entirely to a study of the method published by Paul in 1909 for the determination of mineral oil in turpentine<sup>2</sup>. The study of the Veitch and Donk polymerization method for the determination of mineral oil in turpentine was completed last year, and this method is now an official method<sup>3</sup> of the association. It is generally known as the "38 times normal fuming sulfuric acid method," and it requires a very carefully standardized fuming sulfuric acid of 82.38 per cent total sulfur trioxide content to polymerize the turpentine completely and leave only the mineral oil as a residue.

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<sup>1</sup> For report of Sub-committee C and action of the association, see *This Journal*, 1926, 9: 95.

<sup>2</sup> *J. Ind. Eng. Chem.*, 1909, 1: 27.

<sup>3</sup> *Methods of Analysis*, A. O. A. C., 1925, 408.

The value of the results with the fuming sulfuric acid method depends so largely upon the skill and experience of the analyst in proper manipulation and upon the proper strength of the acid used, which must be held within very narrow limits of concentration, that it was felt that the Paul method, which uses ordinary concentrated sulfuric acid and fuming nitric acid of usual strength, should be further studied. If satisfactory results are obtained it should be adopted as an alternative official method for use when only occasional analyses of turpentine must be made, although it requires more time to make the actual test than the method requiring the fuming sulfuric acid.

The method as used this year was modified slightly from the directions as sent out last year. It has been published in the report of the Committee on Changes in Methods<sup>1</sup>.

This method has been studied by the analysts collaborating on turpentine for several years, but it never gave results so nearly uniform and correct as were obtained this year. The several minor changes in the directions as sent out this year were made to simplify and shorten the method. Heretofore 100 cc. of the turpentine sample was taken for the analysis, and double the quantities of other reagents and practically twice the time to run the test were required. A preliminary steam distillation was omitted; it had been included in the original method merely to insure that any heavy mineral oil present, which might not be completely volatile with steam after the sulfonation, would be included in the final oil when treated with the fuming nitric acid. Such heavy oils are not likely to find their way into turpentine, since mineral oils used to adulterate turpentine must be light and volatile. By making a preliminary evaporation spot test on white paper the analyst can tell whether non-volatile oils are present and these are not likely to be mineral oil. Any other than mineral oil would be destroyed by the fuming nitric acid.

A series of three samples was sent to each of eight collaborators, and reports were received from seven. The results are shown in Table 1.

#### DISCUSSION.

Sample No. 1 was pure gum spirits of turpentine. Sample No. 2 was gum turpentine containing 5.0 per cent of added mineral paint thinner. Sample No. 3 was the same gum turpentine with 15.0 per cent of added mineral paint thinner.

As will be noted, the results with one or two exceptions are uniform, consistent, and close to theoretical—as close as can be obtained on a substance like turpentine. The refractive index on Sample No. 1 reported by Collaborator No. 6 would seem to be in error, since neither pure turpentine nor any polymerization or nitration product therefrom

<sup>1</sup> *This Journal*, 1926, 9: 55.

TABLE 1.

COLLABORATOR	SAMPLE NO. 1		SAMPLE NO. 2		SAMPLE NO. 3	
	Residue	Refractive Index	Residue	Refractive Index	Residue	Refractive Index
	<i>per cent</i>		<i>per cent</i>		<i>per cent</i>	
1	0.0	.....	4.6	1.4384	12.4	1.4270
2	0.2	.....	3.6	1.4300	12.0	1.4192
3	0.0	.....	3.8	1.435	13.2	1.4301
			4.0		12.8	1.4309
4	0.0	.....	1.6	1.4266	10.9	1.4270
5	0.0	.....	8.0	1.4420	14.6	1.4310
6	0.8	1.4580	4.7	1.4342	13.6	1.4322
7	0.0	.....	4.8	1.4321	12.8	1.4270

gives such low figures. The refractive index of turpentine usually falls around 1.4710. A trace of residue is sometimes obtained on pure turpentine but always with much higher refractive index.

The results on Sample No. 2, containing 5 per cent of mineral oil, with the exception of the results reported by Collaborator No. 4, are in fairly close agreement with the theoretical for a substance like turpentine, and as close to theoretical as can be obtained with the already official sulfuric acid method.

Except for the results reported by Collaborator No. 4 on Sample No. 3, which are low, the other results are in close agreement.

It is believed that another season's study of this method will bring the collaborators in still closer agreement, and that this method is of value where only occasional analyses of turpentine must be made. It is recommended that the method be further studied next year, with the view to adoption as an alternative official method for determining mineral oil in turpentine<sup>1</sup>.

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The proceedings for Wednesday were published in Vol. IX, No. 1.

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<sup>1</sup>For Report of Sub-Committee B and action of the Association, see *This Journal*, 1926, 9: 75.

## CONTRIBUTED PAPERS.

## NOTE ON THE DIETHYLPHTHALATE TEST.

By H. WALES (Drug Control Laboratory, Bureau of Chemistry, Washington, D. C.).

The usual tests for diethylphthalate depend upon the saponification of the ester and condensation of the resulting o-phthalic acid with a phenol. Both the U. S. Pharmacopeia<sup>1</sup> and the Bureau of Internal Revenue<sup>2</sup> tests for diethylphthalate depend upon the condensation of the o-phthalic acid with resorcinol to produce a dye of high fluorescent power. The only specific reference found in the literature to a test for diethylphthalate in drug products is to the work done by Eilles<sup>3</sup>, who describes a different method and merely mentions that fluidextracts and tinctures should be clarified with lead acetate before the test is made.

Many crude drugs contain derivatives of naphthalene, anthracene, or phenanthrene, all of which may be oxidized to o-phthalic acid. Theoretically such preparations when tested for phthalates might give positive results, as was found to be the case with a number of products believed to be made with pure alcohol. The decomposition of the plant material probably proceeds slowly, as a freshly prepared tincture of hyoscyamus failed to give a positive test for phthalate, while several that had been prepared for some time gave the test. It is apparent, therefore, that some method must be used to remove these interfering substances before the test for phthalic acid derived from diethylphthalate can be made.

On applying the lead acetate clarification to several products, as suggested by Eilles, it was found that positive tests were still obtained in some cases. However, basic lead acetate did remove the interfering substances when used as described and did not remove diethylphthalate from products containing traces of this compound.

The following procedure, which embodies the essential features of the published methods, has been found to give satisfactory results:

## METHOD.

To such a quantity of the preparation as contains about 10 cc. of alcohol add an excess of basic lead acetate solution, U. S. P., and filter; then add an excess of solid sodium carbonate to precipitate the lead, filter, and extract the filtrate with 15-20 cc. of petroleum benzine. Add 0.2 cc. of approximately 10 per cent sodium hydroxide to the benzine extract and evaporate to dryness on the steam bath. Add to the residue 5 cc. of concentrated sulfuric acid and warm on the steam bath for several minutes to convert the phthalic acid to anhydride. Add 25 milligrams of resorcinol and warm to effect solution. Transfer to a clean dry test tube and heat in a paraffin bath at 160°-170°C. for 10 minutes. Pour

<sup>1</sup> U. S. Pharmacopeia X, p. 353.

<sup>2</sup> Appendix to Regulations No. 61. Formulae for completely and specially denatured alcohol. Revised Sept. 21, 1923, p. 13.

<sup>3</sup> *Z. Nahr. Genussm.*, 1923, 45: 379.

the cooled melt into 150 cc. of water and make alkaline with approximately 10 per cent sodium hydroxide. If diethylphthalate is present, a greenish yellow fluorescence appears at once and persists indefinitely without fading.

Wash all glassware with soap and rinse several times with alcohol before using. porcelain dishes are used for the evaporation, heat first to redness. As a precautionary measure, run a blank. In all cases in which fluorescence is observed allow the solutions to stand 36-48 hours before reporting the presence of diethylphthalate, thus guarding against any pseudo fluorescence.

#### DISCUSSION OF RESULTS.

Several of the products examined formed very bad emulsions with petroleum benzine, and some material which gave a pseudo fluorescence that did not fade completely for two to three days was carried through. In these cases it was found necessary to make a preliminary distillation. The distillate containing the alcohol, diethylphthalate, and other volatile substances present was then treated with basic lead acetate and subjected to the test. In fact, in every instance where a positive test is obtained the writer believes that as an additional precaution the result should be confirmed by distilling some of the product under examination and repeating the test.

The following fluidextracts and tinctures, many of which give positive tests for phthalates by the usual methods, have been examined by the proposed method with negative results; belladonna, black haw, buchu, cascara sagrada, cinchona, colchicum, digitalis, gelsemium, hyoscyamus, ipecac, licorice, nux vomica, senega, and squill.

#### SUMMARY.

A fluorescence obtained from drug products by either the petroleum benzine extraction method or the distillation method does not prove the presence of diethylphthalate unless interfering substances are removed by means of basic lead acetate. Even then a positive result, when obtained by the extraction method, should be confirmed by distilling some of the product under examination and applying the test on the distillate after first treating it with basic lead acetate.

#### THE IODINE NUMBER OF PAPRIKA OIL.

By LLOYD C. MITCHELL (U. S. Food and Drug Inspection Station<sup>1</sup>, St. Louis, Mo.).

The iodine number of the non-volatile ether extract of paprika was used by Doolittle and Ogden as a means of detecting the addition of olive oil to the ground product<sup>2</sup>. A. L. Winton<sup>3</sup>, Associate Referee on Spices in 1908, showed that very erratic results were obtained when a

<sup>1</sup> Ernest R. Smith, Chief.

<sup>2</sup> *J. Am. Chem. Soc.*, 1908, 30: 1481.

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 122, p. 35.

determination of the iodine number was made on the dried oily residue remaining from the determination of ether extract or crude fat by the official method<sup>1</sup>. He believed that the method of securing the non-volatile ether extract for the determination of the iodine number was seriously at fault and that satisfactory results could be obtained only by a purely conventional method. W. Denis<sup>2</sup> found that it was impossible to obtain portions of oil of identical composition when the paprika was extracted with either ordinary ether or petroleum ether (b. p. 50°–60°C.) and also experienced difficulty in obtaining good duplicate determinations by the Doolittle-Ogden cold anhydrous ether extraction method. She then modified the method to provide for extracting larger quantities of paprika, making up the extract to a definite volume with chloroform after drying to constant weight at 100°C., and using aliquot portions for the iodine number.

A. F. Seeker<sup>3</sup>, Associate Referee on Spices in 1909, and again in 1910, also modified the Doolittle-Ogden method. Seeker's modification was adopted by the association as a provisional method and designated a "Method for the detection of olive oil in paprika". No further reports on Seeker's method<sup>4</sup> were published by the association, and it is now a tentative method.

Because all the investigators have shown that the oil extracted from paprika is unstable, more than ordinary precautions must be taken in order to avoid oxidation and the consequent lowering of the iodine value.

To avoid the possibility of error due to drying the paprika, heating the extracted oil, or exposing the dried oil to the atmosphere, and to eliminate the elaborate precautions which Seeker recommended, a new method was worked out. This method consists essentially in extracting the paprika with chloroform, drying one portion for the determination of the oil content, and using the other portion for the determination of the quantity of iodine absorbed.

The work described compares the present tentative method, which is Seeker's modification of the anhydrous ether extraction method, with the chloroform extraction method.

The details of the chloroform extraction method are as follows:

#### METHOD.

Transfer 10 grams of the ground sample to a 100 cc. volumetric flask, add 50–75 cc. of chloroform, and let stand for 1 hour, shaking frequently. Make up to the 100 cc. mark with chloroform, mix thoroughly, filter through a 12½ cm. folded filter, make the filtrate up to 100 cc. with chloroform, and again mix thoroughly. Pipet off successively four 20 cc. portions of the solution, using the same pipet. Transfer two of the 20 cc. portions to weighed air-dry 50 cc. Erlenmeyer flasks. Evaporate the solvent by placing

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 117.

<sup>2</sup> U. S. Dept. Agr. Bur. Chem. Bull. 122, p. 213.

<sup>3</sup> U. S. Dept. Agr. Bur. Chem. Bull. 132, p. 112; 137, p. 80.

<sup>4</sup> *Methods of Analysis*, A. O. A. C., 1925, 317.

the flask on a steam bath, tilting the flask at a 45° angle. Dry the flask and contents at 100°C. for 30 minutes by laying the flask on its side and allowing to cool in the air for 30 minutes. Weigh. Repeat the drying, cooling, and weighing until the weight is constant to within 1 mg., two periods of drying usually being sufficient. Note the weight of extract obtained. Transfer the two remaining 20 cc. portions to suitable glass-stoppered flasks or bottles for the determination of the iodine number. Add 30 cc. of Hanus solution and follow the official method<sup>1</sup>.

NOTE: The filtrate was made up to 100 cc. in this work in order to provide sufficient volume for a number of aliquots, as in the routine examination of paprika the writer prefers to use separately prepared samples for duplicate determinations. Each 10 gram sample in a 200 cc. glass-stoppered flask is treated with 100 cc. of chloroform added from a pipet, the mixture is allowed to stand 1 hour with occasional shaking, the solution is filtered through a 12½ cm. folded filter, and two 20 cc. portions are withdrawn. (As in the case with ether, prolonged extraction with chloroform causes the solution of difficultly soluble substances, probably resins, which have a lower iodine value. Successive 100 cc. washings showed lower iodine numbers, the third 100 cc. portions having iodine values as low as 70. Whether this was due to the nature of the dissolved matter or to other causes is difficult to determine.) One of the 20 cc. portions is used for the determination of the oil content by evaporating the solvent in a crystallizing dish, 50 x 35 mm., on a steam-bath, the dish and contents are dried at 100°C. for 1 hour, cooled in the air for 30 minutes, and weighed, then dried for 30 minutes, cooled, and weighed. This process is repeated until the weight is constant to within 1 mg., although two periods of drying are usually sufficient. The other 20 cc. portion is used for the direct determination of the iodine absorption value.

#### DISCUSSION OF RESULTS.

The results obtained by the anhydrous ether extraction method and the chloroform extraction method are found in Tables 1 and 2. The weight of the non-volatile ether extract obtained by the official continuous extraction method<sup>2</sup> on 2.0 grams of the sample is given. The anhydrous ether extraction method used in preparing the samples for the iodine number yields from 0.0228 to 0.0472 gram less extract for 2.0 gram samples. The weight of extract shown by the chloroform extraction method is not comparable to the other two weights of extracts because no allowance was made for the volume occupied by the sample and the filtrate was again made to 100 cc.

The samples of Hungarian paprika reported on in Table 1 were received under the seal of the Royal Hungarian Ministry of Agriculture through the Department of State. They consisted of large wreaths, the pods of which were of a bright red color; they were sound, from 6 to 10 cm. in length and from 2.5 to 4 cm. in diameter, and of a conical shape.

The samples of Spanish paprika were received under seal from the American Consul General at Barcelona, Spain. They also consisted of large wreaths, the pods of which were of a bright red color; they were sound, from 2.5 to 4 cm. in length and from 4 to 6 cm. in diameter, and were nearly round.

It will be noted in the tables that samples extracted with chloroform showed the same, or slightly lower, iodine values when run directly as when run on the dried oil. The average weight of oil obtained on drying

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 287.

<sup>2</sup> *Ibid.*, 319.



TABLE 1.  
*Hungarian paprika.*

SAMPLE NUMBER	NON- VOLATILE ETHER EXTRACT	ANHYDROUS ETHER EXTRACTION METHOD		CHLOROFORM EXTRACTION METHOD		
		WEIGHT OF EXTRACT	IODINE NUMBER	WEIGHT OF EXTRACT	Iodine Number	
					On Weighed Portion	Direct
	gram	gram		gram		
Whole pods including stems—usual grade.						
1	0.2440	0.2162	134.2	0.1752	136.2	136.2
	0.2444	0.2180	133.7	0.1756	136.2	136.2
2	0.2656	0.2416	131.2	0.1886	134.9	134.9
	0.2652	0.2436	131.7	0.1886	136.7	134.9
3	0.2596	0.2286	133.7	0.1850	135.8	135.8
	0.2628	0.2306	133.0	0.1858	135.8	134.5
4	0.3016	0.2754	129.3	0.2188	134.0	133.5
	0.3026	0.2726	130.2	0.2192	134.6	133.5
5	0.2880	0.2556	133.2	0.2052	133.6	132.2
	0.2906	0.2584	132.8	0.2052	133.6	132.2
6	0.3288	0.2998	132.6	0.2444	134.3	132.6
	0.3284	0.3002	132.9	0.2448	134.3	132.1
7	0.2734	0.2442	133.7	0.1898	135.4	134.1
	0.2760	0.2450	133.8	.....	....	133.4
Maximum	0.3288	0.3002	134.2	0.2448	136.7	136.2
Minimum	0.2440	0.2162	129.3	0.1752	133.6	132.1
Average	0.2808	0.2511	132.6	0.2011	135.0	134.0
Whole pods excluding stems—choice grade.						
8	0.2790	0.2524	133.0	0.2116	135.5	135.5
	0.2788	0.2530	132.2	0.2118	135.5	135.5
9	0.2928	0.2530	132.7	0.2076	134.0	134.0
	0.2910	0.2542	132.6	0.2072	135.2	134.6
10	0.2846	0.2612	128.9	0.2008	135.6	134.9
	0.2846	0.2570	130.0	0.2014	135.6	134.9
11	0.3234	0.3016	129.3	0.2474	133.2	132.7
	0.3256	0.3016	128.8	0.2476	132.7	132.7
12	0.3176	0.2816	132.9	0.2240	134.5	133.9
	0.3170	0.2826	133.4	.....	.....	133.9
13	0.3466	0.3038	133.0	0.2418	134.7	133.6
	0.3472	0.3042	132.8	0.2422	134.7	133.0
14	0.2948	0.2584	133.3	0.2178	134.0	132.8
	0.2942	0.2598	133.0	0.2182	133.4	132.2
Maximum	0.3472	0.3042	133.4	0.2476	135.6	134.9
Minimum	0.2788	0.2524	128.8	0.2008	132.7	132.2
Average	0.3055	0.2746	131.8	0.2217	134.5	133.9

duplicate portions as the basis of calculation was used. These lower values might possibly be due to the presence of a very small quantity of volatile extractive material. Although the results are not reported in this paper, it may be stated that the samples of Hungarian paprika gave from 1 to 21 mg. of volatile ether extract on a 2.0 gram sample as determined by the official continuous ether extraction method. The samples of Spanish paprika showed from 1 to 11 mg. of volatile material for a 2.0 gram sample.

TABLE 2.  
*Spanish paprika.*

SAMPLE NUMBER	NON- VOLATILE ETHER EXTRACT	ANHYDROUS ETHER EXTRACTION METHOD		CHLOROFORM EXTRACTION METHOD		
		Weight of Extract	Iodine Number	Weight of Extract	Iodine Number	
					On Weighed Portion	Direct
	gram	gram		gram		
Whole pods including stems—usual grade.						
1	0.2520	0.2150	136.1	0.1846	134.8	133.4
	0.2512	0.2174	135.8	0.1854	135.5	133.4
2	0.2340	0.2064	136.8	0.1834	133.2	133.2
	0.2348	0.2078	137.7	0.1832	133.2	133.2
3	0.2330	0.1956	136.4	0.1750	134.1	133.2
	0.2322	0.1968	136.2	0.1750	134.1	133.2
4	0.2518	0.2062	137.5	0.1816	133.7	133.7
	0.2514	0.2082	136.9	0.1818	133.7	133.7
Maximum	0.2520	0.2174	137.7	0.1854	135.5	133.7
Minimum	0.2322	0.1956	135.8	0.1750	133.2	133.2
Average	0.2426	0.2067	136.7	0.1825	134.0	133.4
Whole pods excluding stems—choice grade.						
5	0.2476	0.2136	137.1	0.1912	135.2	134.5
	0.2470	0.2158	136.9	0.1912	136.6	133.9
6	0.2438	0.2094	137.4	0.1930	134.4	133.7
	0.2408	0.2118	137.0	0.1930	134.4	134.4
7	0.2576	0.2252	134.6	0.1932	134.2	133.4
	0.2684	0.2266	134.3	0.1936	134.2	133.4
8	0.2666	0.2188	136.1	0.1966	134.0	133.2
	0.2668	0.2202	135.8	0.1968	134.0	133.2
9	0.2324	0.1958	135.6	0.1792	133.8	132.9
	0.2308	0.1960	135.5	0.1798	133.8	132.9
Maximum	0.2684	0.2266	137.4	0.1968	136.6	134.5
Minimum	0.2308	0.1958	134.3	0.1792	133.8	132.9
Average	0.2502	0.2133	136.0	0.1908	135.5	133.6

The samples of Hungarian paprika yielded iodine values somewhat higher by the chloroform extraction method than by the anhydrous ether extraction method, but the reverse was true of the samples of Spanish paprika.

#### SUMMARY.

The chloroform extraction method appears to have the following advantages over the anhydrous ether extraction method:

- (1) It is not necessary to dry the sample over sulfuric acid.
- (2) It is not necessary to prepare anhydrous alcohol-free ether.
- (3) The quantity of iodine absorbed can be determined on one portion while the weight of oil used to calculate the iodine number is obtained on another portion. The actual time required to determine the iodine number is from one-half to one-third of the time required to determine the iodine value by the present tentative method.
- (4) The possibility of oxidation of the oil is reduced to a minimum.
- (5) It yields results that are in close agreement with the anhydrous ether extraction method.

#### NOTE ON INFLUENCE OF PEPTIC DIGESTION IN THE DETERMINATION OF TOTAL CARBOHYDRATES IN CEREAL PRODUCTS.

By B. G. HARTMANN and FRED HILLIG (Food Control Laboratory, Bureau of Chemistry, Washington, D. C.).

No official method is available for the determination of starch in cereal products. The general method for the determination of the starch content of products containing large quantities of proteinaceous matter by conversion into dextrose by means of diastase and acid hydrolysis is tedious and at times unreliable. It was believed by the authors that the difficulty of the conversion is attributable to the occlusion of starch by the proteins. It was observed that when graham flour was digested with pepsin overnight, conversion of starch with a malt infusion was complete in one-half hour. As this observation seemed significant, the following procedure was tried: One-tenth gram of pepsin in a 250 cc. beaker was dissolved in 50 cc. of tap water. Two grams of the material was added, stirred vigorously, and allowed to stand at room temperature overnight. The mixture was then heated to boiling for one minute, stirred constantly, cooled to 65°C., and held at this temperature for 15 minutes. After 20 cc. of malt infusion had been added, the temperature of 65°C. was maintained for 1 hour longer. Generally, conversion was then complete, as shown by the iodine test. In cases where the iodine test showed unconverted starch, the mixture was again heated to boiling, cooled to 65°C., and after the addition of 20 cc. more of the malt infusion, held at

65°C. until conversion was complete. The dextrose was determined by the Munson-Walker method<sup>1</sup>. A blank was carried through on the same volume of malt infusion used in the determination. The approximate carbohydrate content was obtained by multiplying the difference between the weight of dextrose given by the sample and that given by the blank by the factor 0.9. The results for the carbohydrate content calculated in this way are of course not more than approximate, except in the absence of sugars in the original sample.

Before trying the method on cereal products, determinations were made on starches from different sources, with the following results:

	WHEAT STARCH	CORN STARCH	POTATO STARCH
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
	82.9	85.6	84.2
	83.6	85.3	83.9
	84.3	86.9	84.4
	84.1	85.9	85.4
Carbohydrate, average .	83.73	85.93	84.48
Moisture . . . . .	9.91	10.93	13.92
Protein . . . . .	0.40	0.40	0.14
Fat . . . . .	0.41	0.43	0.12
Ash . . . . .	0.10	0.13	0.24
Total . . . . .	94.55	97.82	98.90

In explanation of the low "total" obtained on the wheat starch, it might be stated that conversion was not complete, as indicated by the iodine test, as for some unknown reason the material did not gelatinize properly. The potato and corn starches gelatinized readily, and conversion with malt infusion was complete in one-half hour.

The following results were obtained on flours, semolinas, graham flours, and<sup>1</sup>gluten flour:

	CARBOHYDRATE <i>per cent</i>
Patent flour . . . . .	75.4 75.1
Clear flour . . . . .	73.2 72.7
Semolina No. 3 . . . . .	69.7 68.9
Semolina No. 1 . . . . .	71.5 70.9
Graham flour . . . . .	60.8 61.0
Graham flour . . . . .	64.8 65.2
Gluten flour . . . . .	38.3 38.9 38.4

<sup>1</sup> *Methods of Analysis*, A. O. A. C., 1925, 190.

These results show that a digestion with pepsin is helpful. If it is utilized it might lead to a reliable method for the determination of starch in cereal and other products high in protein. In all cases the conversion was complete in one-half hour, which is particularly gratifying in the case of the gluten flour because of its high protein content. The determinations were made on the *original unground* sample, and this fact may account for the poor checks in some instances.

# INDEX TO VOLUME IX.

PROCEEDINGS OF THE FORTY-FIRST ANNUAL CONVENTION, 1925.

	PAGE
Acetic acid, combined, method for determination.....	49
Acetysalicylic acid	
changes in methods.....	48
method for determination.....	49
in mixtures containing acetphenetidin and caffeine.....	50
recommendations	
by Committee B.....	76
by Harrison.....	281
report	
by Harrison.....	278
by Paul.....	267
Acid hydrolysis method for fat in eggs and egg products.....	58
Acidity of highly colored fruit-type products, method for determination, paper by Badger and Sale.....	342
Address	
by Browne, President.....	15
by Jardine, Secretary of Agriculture.....	460
by Wiley, Honorary President.....	382
Adulteration of stock feed	
recommendations	
by Committee A.....	72
by Gensler.....	153
report by Gensler.....	148
Agricultural chemical analysis, Purposes and aims, address by President Browne..	15
Alcohol in drug products	
method.....	285
recommendations	
by Committee B.....	76
by Lynn.....	285
report	
by Lynn.....	282
by Paul.....	267
Alcohol and specific gravity	
recommendations	
by Committee B.....	75
by Hann.....	440
report by Hann.....	437
Alfend, report on fat (by acid hydrolysis) lipoids and lipoid-phosphoric acid ( $P_2O_5$ ) and water-soluble protein-nitrogen precipitable by 40 per cent alcohol in flour.....	429
Alfend and Mitchell, paper, analysis of butter.....	209
Alfend, Smith and Mitchell, paper, detection of added pepper shells in pepper...	333
Alimentary pastes	
changes in methods.....	43
method for determination	
of protein.....	44
of total solids (indirect method).....	43
recommendations by Committee C.....	92
wheat flour, and eggs, quantitative determination of unsaponifiable matter, paper by Hertwig and Bailey.....	122
Alkaloids, ipecac	
recommendations	
by Bliss.....	306
by Committee B.....	77
report by Bliss.....	301

	PAGE
Ammoniacal and organic nitrogen, eggs and egg products, method.....	57
Analysis of butter, paper by Mitchell and Alfend.....	209
Apomorphine	
recommendations by Committee B.....	79
report	
by Glycart.....	323
by Paul.....	275
Arsenic in sodium cacodylate	
method for determination.....	51, 287
recommendations by Glycart.....	288
report by Glycart.....	286
Arsenicals	
changes in methods.....	51
recommendations	
by Committee B.....	76
by Glycart.....	288
report	
by Glycart.....	286
by Paul.....	269
Ash	
in bread.....	
in flour	
recommendations by Mangels.....	415
report by Mangels.....	409
in fruit products, recommendations by Committee C.....	86
Atophan, report by Paul.....	277
Auditing committee.....	180
report by McDonnell.....	111
Badger, report, waters, brine and salt.....	125
Badger and Sale, paper, method for determination of acidity of highly colored fruit-type products.....	342
Bailey, C. H., report on hydrogen-ion concentration of flour.....	433
Bailey, E. M., report on cacao products.....	461
Bailey, L. H., report	
baking powder.....	253
methods for examination of bread.....	426
Bailey and Hertwig, paper, quantitative determination of unsaponifiable matter in wheat flour, alimentary pastes, and eggs.....	122
Baked cereal products	
changes in methods.....	42
recommendations by Committee C.....	91
Baking powder	
determination of fluorides	
recommendations	
by Committee C.....	82
by Morton.....	265
report by Morton.....	262
recommendations by Bailey.....	262
report by Bailey.....	253
Baking powders and baking chemicals	
changes in methods.....	45
recommendations by Committee C.....	81
Balcom, report	
of board of editors.....	59
on publications, Oct. 1, 1924 to Oct. 15, 1925.....	62
Barbital and phenobarbital	
method for determination.....	51
recommendations by Committee B.....	79
report by Paul.....	276
Barley, comparison of commercial grading with macroscopical and chemical analysis, paper by Mitchell.....	197
Bartlett, obituary on Charles Dayton Woods.....	No. 3, iii
Berry, report on gelatin.....	458

<b>Beverages, non-alcoholic, and flavors</b>	
recommendations	
by Committee C.....	87
by Sale.....	456
report by Sale.....	446
<b>Bibliography, report of Committee (Skinner).....</b>	<b>110</b>
<b>Bio-assay of drugs</b>	
recommendations by Committee B.....	79
report	
by Paul.....	276
by Schwartz.....	330
<b>Bitter tonics and laxatives</b>	
recommendations by Committee B.....	77
report by Fuller.....	306
<b>Blanck, report, committee on sampling.....</b>	<b>107</b>
<b>Blish, report on glutenin in wheat flour.....</b>	<b>417</b>
<b>Bliss, report, ipecac alkaloids.....</b>	<b>301</b>
<b>Board of editors, report by Balcom.....</b>	<b>59</b>
<b>Bopst, report, feeding stuffs.....</b>	<b>146</b>
<b>Bread</b>	
method for determination	
of ash.....	42
of protein.....	42
of total solids in air-dried ground sample and entire loaf.....	42
recommendations by Bailey.....	429
report by Bailey.....	426
preparation of sample.....	42
<b>Brine, waters, and salt</b>	
changes in methods.....	29
recommendations	
by Badger.....	126
by Committee A.....	70
report by Badger.....	125
<b>Browne, president's address.....</b>	<b>15</b>
<b>Burgess, report, reaction value of soils.....</b>	<b>138</b>
<b>Butter</b>	
analysis, paper by Mitchell and Alfend.....	209
report by Hortvet.....	231
<b>Cacao butter, recommendations by Committee C.....</b>	<b>95</b>
<b>Cacao products</b>	
changes in methods.....	46
determination of fat by Lepper-Waterman method.....	46
recommendations	
by Bailey.....	473
by Committee C.....	95
report by Bailey.....	461
<b>Camphor</b>	
changes in methods.....	52
method for determination.....	52
report by Paul.....	269
<b>Camphor and monobromated camphor</b>	
recommendations by Committee B.....	77
report by Eaton.....	288
<b>Canned foods, recommendations by Committee C.....</b>	<b>86</b>
<b>Carbohydrates, total, in cereal products, influence of peptic digestion in determination, paper by Hartmann and Hillig.....</b>	<b>482</b>
<b>Carbon tetrachloride, recommendations by Committee B.....</b>	<b>77</b>
<b>Carbon tetrachloride and chloroform</b>	
method for estimation.....	300
recommendations by Moraw.....	300
report	
by Moraw.....	296
by Paul.....	270



	PAGE
Casein in milk, paper by Waterman.....	246
Cereal foods	
changes in methods.....	39
recommendations	
by Committee C.....	88
by Hertwig.....	400
report by Hertwig.....	389
Cereal products	
baked	
changes in methods.....	42
recommendations by Committee C.....	91
influence of peptic digestion in determination of total carbohydrates, paper by Hartmann and Hillig.....	482
Changes in official and tentative methods of analysis made at forty-first annual convention.....	27
Chaulmoogra oil	
method for determination.....	52
recommendations	
by Committee B.....	77
by Warren.....	295
report	
by Paul ..	269
by Warren.....	290
Cheese	
determination of moisture.....	44
recommendations by Mitchell.....	243
report by Mitchell.....	238
recommendations by Committee C.....	80
report by Hortvet.....	232
Chemical methods for reducing sugars	
recommendations	
by Committee A.....	73
by Jackson.....	178
report by Jackson.....	178
Chemical reagents, testing	
recommendations by Committee B.....	75
report by Spencer.....	347
Chloramine products	
recommendations	
by Committee B.....	77
by Jones.....	296
report	
by Jones.....	295
by Paul.....	270
Chloroform and carbon tetrachloride	
method for estimation.....	300
recommendation	
by Committee B.....	77
by Moraw.....	300
report	
by Moraw.....	296
by Paul.....	270
Cinchophene, report by Paul.....	277
Clark and Dill, paper, formaldehyde in certain marine products.....	117
Clarke, report	
on metals in foods.....	364
on vinegars.....	440
Coe, report, method for determining starch in presence of interfering polysaccharides.....	147
Collection and preparation of sample	
alimentary pastes.....	43
eggs and egg products.....	56

	PAGE
Coloring matters in foods	
recommendations	
by Committee C.....	84
by Jablonski.....	364
report by Jablonski.....	362
Combined acetic acid, method for determination.....	49
Committee A on recommendations of referees, report by Ross.....	70
B on recommendations of referees, report by Lythgoe.....	75
C on recommendations of referees, report by Doolittle.....	79
on auditing.....	180
report by McDonnell.....	111
on bibliography, report by Skinner.....	110
on definitions of terms and interpretation of results on fertilizers, report by Haskins.....	66
on editing methods of analysis, report by Doolittle.....	25
on nominations.....	180
report by Veitch.....	111
on recommendations of referees, report by Doolittle.....	69
on resolutions.....	180
report by Fraps.....	112
on revision of methods of soil analysis, report by MacIntire.....	69
on sampling, report by Blanck.....	107
to consider advisability of studying methods for analysis of paint, report by Hand.....	107
to cooperate in revision of United States Pharmacopeia, report by Kebler.....	95
to cooperate with other committees on food definitions, report by Hortvet.....	103
Committees named by president.....	180
officers, referees and associate referees for year ending October, 1926.....	1
Comparison of commercial grading of barley with macroscopical and chemical analysis, paper by Mitchell.....	197
Condiments and spices, recommendations by Committee B.....	75
Corrections for Volume IX, No. 1.....	196
Cream and milk, report by Hortvet.....	233
Crop Protection Institute of National Research Council, report of representatives Hartwell and Patterson.....	97
Crude fiber, recommendations by Committee C.....	95
Dairy products	
changes in methods.....	44
recommendations	
by Committee C.....	79
by Hortvet.....	237
report by Hortvet.....	231
Densimetric, drying, and refractometric methods for sugar-house products	
recommendations by Committee A.....	73
report by Schneller.....	156
Detection and determination of lactic acid in presence of other organic acids, paper by Nelson.....	331
Diethylphthalate test, paper by Wales.....	476
Dill and Clark, paper, formaldehyde in certain marine products.....	117
Diversifying the uses of farm crops, address by Secretary of Agriculture Jardine.....	460
Doolittle, R. E.	
announcement of death.....	No. 2, vi
report of committee	
on editing methods of analysis.....	25
on recommendations of referees.....	69
C on recommendations of referees.....	69
Dried milk, determination of moisture, report by Keister.....	243
Dried milk and malted milk	
recommendations by Committee C.....	80
report by Hortvet.....	236
Drug section.....	266

	PAGE
<b>Drugs</b>	
changes in methods	48
recommendations	
by Committee B	76
by Paul	277
report by Paul	266
<b>Drying, densimetric and refractometric methods for sugar-house products</b>	
recommendations by Committee A	73
report by Schneller	156
<b>Dunlap, presentation of address by Secretary Jardine</b>	460
<b>Eaton, report, camphor and monobromated camphor—estimation of camphor in pills and tablets</b>	288
<b>Editing methods of analysis, report of committee by Doolittle</b>	25
<b>Editors, report of board by Balcom</b>	59
<b>Eggs, alimentary pastes, and wheat flour, quantitative determination of unsaponifiable matter, paper by Hertwig and Bailey</b>	122
<b>Eggs, liquid and powdered, determination of total solids</b>	
recommendation by Palmer	357
report by Palmer	354
<b>Eggs and egg products</b>	
changes in methods	56
recommendations	
by Committee C	82
by Hertwig	351
report by Hertwig	348
<b>Electrolytic deposition from sulfuric and nitric acid solution, method for determination</b>	38
<b>Elliott, report, separation of quinine and strychnine</b>	310
<b>Elmore, paper, determination of strychnine in poisoned grains</b>	224
<b>Emery, report of representative at National Conference of Pharmaceutical Research</b>	24
<b>Emulsions, mineral oil-soap, methods for determination in insecticides and fungicides</b>	28
<b>Ether</b>	
recommendations by Committee B	79
report by Paul	276
<b>Examination of whole grain, recommendation by Committee A</b>	71
<b>Farm crops, diversifying the uses of, address by Secretary of Agriculture Jardine</b>	460
<b>Fat</b>	
in eggs and egg products	58
in flour	
method for determination	41
recommendations by Alfend	432
report by Alfend	429
<b>Fats and oils</b>	
changes in methods	44
determination of unsaponifiable residue	45
recommendations	
by Committee C	81
by Jamieson	253
report by Jamieson	247
<b>Feeding stuffs</b>	
changes in methods	30
recommendations by Committee A	71
report by Bopst	146
<b>Feeds</b>	
cattle, moisture content, recommendations by Committee A	72
mineral mixed, recommendations by Committee A	72
<b>Fertilizers</b>	
changes in methods	27
definition of terms and interpretation of results, report of committee, by Haskins	66

	PAGE
<b>Fertilizers—Continued</b>	
mixed, determination of potash content, paper by Fraps.....	192
recommendations	
by Committee A.....	74
by Fraps.....	182
report by Fraps.....	181
<b>Financial report</b>	
of Secretary-Treasurer, October 1, 1924 to October 15, 1925, by Skinner.....	64
on publications, October 1, 1924 to October 15, 1925, by Balcom.....	62
<b>Flavoring extracts</b>	
changes in methods.....	47
determination of lead number by Wichmann method.....	47
method for determination of vanilla resins in vanilla.....	48
<b>Flavors and non-alcoholic beverages</b>	
recommendations	
by Committee C.....	87
by Sale.....	456
report by Sale.....	446
<b>Flour</b>	
ash content	
recommendations by Mangels.....	415
report by Mangels.....	409
determination of total solids.....	39
directions for sampling.....	39
fat content	
recommendations by Alfend.....	432
report by Alfend.....	429
hydrogen-ion concentration	
recommendations by Bailey.....	436
report by Bailey.....	433
moisture content, report by Spencer.....	404
recommendations by Committee C.....	88
sampling	
recommendation by Runkel.....	426
report by Runkel.....	423
wheat, glutenin content	
recommendation by Blish.....	423
report by Blish.....	417
<b>Fluorides in baking powders</b>	
recommendations	
by Committee C.....	82
by Morton.....	265
report by Morton.....	262
<b>Food definitions, report of committee to cooperate with other committees, by Hortvet.....</b>	103
<b>Food preservatives</b>	
recommendations	
by Committee C.....	84
by Randall.....	361
report by Randall.....	358
<b>Foods</b>	
canned, recommendations by Committee C.....	86
cereal	
recommendations	
by Committee C.....	88
by Hertwig.....	400
report by Hertwig.....	389
coloring matter content	
recommendations	
by Committee C.....	84
by Jablonski.....	364
report by Jablonski.....	362
metal content	
recommendations	
by Clarke.....	366
by Committee C.....	85
report by Clarke.....	364

	PAGE
Formaldehyde in certain marine products, paper by Dill and Clark.....	117
Formic acid, separation in food products by distillation with xylene, paper by Morton and Spencer.....	221
Fraps	
paper, method for potash in mixed fertilizers.....	192
report	
committee on resolutions.....	112
fertilizers.....	181
Fruit acids	
recommendations by Committee C.....	85
report by Nelson.....	375
Fruit products, ash content, recommendations by Committee C.....	86
Fruit-type products, highly colored, method for determination of acidity, paper by Badger and Sale.....	342
Fruits and fruit products	
changes in methods.....	38
recommendations	
by Committee C.....	85
by Gowen.....	373
report by Gowen.....	367
Fuller, report, laxatives and bitter tonics.....	306
Fungicides and insecticides	
changes in methods.....	27
recommendations	
by Committee A.....	71
by Graham.....	137
report by Graham.....	127
Gelatin	
recommendations	
by Berry.....	459
by Committee C.....	88
report by Berry.....	458
Gensler, report, stock feed adulteration.....	148
Glutenin in wheat flour	
recommendation by Blish.....	423
report by Blish.....	417
Glycart, report	
on apomorphine hydrochloride.....	323
on arsenicals.....	286
Gowen, report, fruits and fruit products.....	367
Graham, report, insecticides and fungicides.....	127
Grain, whole, examination of, recommendation by Committee A.....	71
Grains, poisoned, determination of strychnine content, paper by Elmore.....	224
Grape juice	
determination of added water, recommendations by Committee C.....	85
report by Hartmann.....	374
white, method for determination of added water.....	38
Gravimetric determination of phosphoric acid, report by Ross.....	182
Grotlich, report on turpentine.....	473
Hand, report, committee to consider advisability of studying methods for analysis of paint.....	107
Hann, report on specific gravity and alcohol.....	437
Hanson, A. W., report, nitroglycerin.....	316
Hanson, H. H., obituary on Charles Lyndall Penny.....	No. 4, iii
Harrison, report, acetylsalicylic acid.....	278
Hartmann, report on added water in grape juice.....	374
Hartmann and Hillig, paper, note on influence of peptic digestion in determination of total carbohydrates in cereal products.....	482
Hartwell and Patterson, report of board of governors of Crop Protection Institute of National Research Council.....	97
Haskins, report of committee on definitions of terms and interpretation of results on fertilizers.....	66

	PAGE
Hertwig, report	
on cereal foods . . . . .	389
on eggs and egg products . . . . .	348
Hertwig and Bailey, paper, quantitative determination of unsaponifiable matter	
in wheat flour, alimentary pastes, and eggs . . . . .	122
Hillig and Hartmann, paper, note on influence of peptic digestion in determina-	
tion of total carbohydrates in cereal products . . . . .	482
Hitchcock, report, silver proteinates . . . . .	312
Hortvet, report	
of committee to cooperate with other committees on food definitions . . . . .	103
on dairy products . . . . .	231
Hydrogen sulfide in waters, method for determination . . . . .	29
Hydrogen-ion concentration of flour	
recommendations by Bailey . . . . .	436
report by Bailey . . . . .	433
Ice cream	
recommendations by Committee C . . . . .	80
report by Hortvet . . . . .	237
Influence of peptic digestion in determination of total carbohydrates in cereal prod-	
ucts, paper by Hartmann and Hillig . . . . .	482
Inorganic plant constituents	
recommendations by Committee A . . . . .	75
report by Patten . . . . .	193
Insecticides and fungicides	
changes in methods . . . . .	27
recommendations	
by Committee A . . . . .	71
by Graham . . . . .	137
report by Graham . . . . .	127
International Congress of Soil Science, announcement by Lipman . . . . .	367
Iodine number of paprika oil, paper by L. C. Mitchell . . . . .	477
Ipecac alkaloids	
recommendations	
by Bliss . . . . .	306
by Committee B . . . . .	77
report	
by Bliss . . . . .	301
by Paul . . . . .	271
Ipecac, fluidextract, method for determination . . . . .	271
Jablonski, report on coloring matter in foods . . . . .	362
Jackson, report, chemical methods for reducing sugars . . . . .	178
Jamieson, report, fats and oils . . . . .	247
Jardine, Secretary of Agriculture, address, diversifying the uses of farm crops . . . . .	460
Jones, report, chloramine products . . . . .	295
Kebler, report of committee to cooperate in revision of U. S. Pharmacopeia . . . . .	95
Keister, report, moisture in dried milk . . . . .	243
Kerr, A. P., report, potash . . . . .	191
Kerr, R. H., report, meat and meat products . . . . .	457
Lactic acid, detection and determination in presence of other organic acids . . . . .	331
Ladd, Edwin Fremont, obituary by Wiley . . . . .	No. 1, iii
Lancaster, report, maple products . . . . .	155
Lane-Eynon general volumetric method . . . . .	35
Latschaw, report, sulfur and phosphorus in the seed of plants . . . . .	194
Laxatives and bitter tonics	
recommendations by Committee B . . . . .	77
report	
by Fuller . . . . .	306
by Paul . . . . .	272
Lead number (Wichmann) method for determination in flavoring extracts . . . . .	47
Leather and tanning materials, report by referee . . . . .	127
Lepper-Waterman method for determination of fat in cacao products . . . . .	46

	PAGE
Liming materials	
recommendations by Shaw.....	144
report by Shaw.....	141
Liming materials and soils	
recommendations by Committee A.....	71
Linseed meal—determination of starch in presence of interfering polysaccharides, recommendations by Committee A.....	72
Lipman, announcement of International Congress of Soil Science.....	367
Lipoid phosphoric acid	
in eggs and egg products.....	59
in flour.....	41
Lipoids and lipoid phosphoric acid	
in eggs and egg products.....	58
method of determination in flour.....	40
Luminal and veronal, report by Paul.....	276
Lynn, alcohol in drug products.....	282
Lythgoe, report of Committee B on recommendations of referees.....	75
MacIntire, report of committee on revision of methods of soil analysis.....	69
McDonnell, report of auditing committee.....	111
Malted and dried milk	
recommendations by Committee C.....	80
report by Hortvet.....	236
Mangels, report on ash in flour.....	409
Maple products	
recommendations	
by Committee A.....	72
by Lancaster.....	156
report by Lancaster.....	155
Marine products, formaldehyde content, paper by Dill and Clark.....	117
Meat and meat products	
recommendations by Committee C.....	88
report by Kerr.....	457
Members and visitors present, 1925 meeting.....	9
Mercurials	
recommendations by Committee B.....	78
report	
by Paul.....	272
by Spencer.....	307
Metals in foods	
recommendations	
by Committee C.....	85
by Clarke.....	366
report by Clarke.....	364
Methods of Analysis, changes made at 1925 meeting.....	27
Methylene blue	
changes in methods.....	53
recommendations by Committee B.....	78
report by Paul.....	276
Microscopical methods, recommendations by Committee C.....	95
Milk	
determination of casein, paper by Waterman.....	246
dried, determination of moisture, report by Keister.....	243
dried and malted	
recommendations by Committee C.....	80
report by Hortvet.....	236
Milk and cream, report by Hortvet.....	233
Mineral mixed feeds, recommendations by Committee A.....	72
Mineral oil in turpentine, sulfuric fuming acid method for determination.....	55
Mineral oil-soap emulsions, methods for determination in insecticides and fungi- cides.....	28
Mitchell	
paper	
comparison of commercial grading of barley with macroscopical and chemi- cal analysis.....	197
iodine number of paprika oil.....	477
report, moisture in cheese.....	238

	PAGE
Mitchell and Alfend, paper, analysis of butter.....	209
Mitchell, Smith and Alfend, paper, detection of added pepper shells in pepper....	333
Moisture	
determination by distillation with toluene	
recommendations by Sterling.....	154
report by Sterling.....	153
in cattle feed, recommendations by Committee A.....	72
in cheese	
method for determination.....	44
recommendation by Mitchell.....	243
report by Mitchell.....	238
in dried milk, report by Keister.....	243
in feeding stuffs, method for determination.....	30
in flour, report by Spencer.....	404
Monobromated camphor	
recommendations by Committee B.....	77
report	
by Eaton.....	288
by Paul.....	269
Moraw, report, chloroform and carbon tetrachloride in drug products.....	296
Morton, report, fluorides in baking powder.....	262
Morton and Spencer, paper, separation of formic acid in food products by distillation with xylene.....	221
Murray, remarks on extraction of fatty acids of soaps.....	137
National Research Council of Crop Protection Institute, report of representatives	
Hartwell and Patterson.....	97
Naval stores, recommendations by Committee B.....	75
Nelson	
paper, detection and determination of lactic acid in presence of other organic acids.....	331
report on fruit acids.....	375
Nitrogen	
in fertilizers, recommendations by Committee A.....	74
organic and ammoniacal, method for determination in eggs and egg products.....	57
recommendations by Prince.....	191
report by Prince.....	187
Nitroglycerin	
recommendations	
by Committee B.....	78
by Hanson.....	323
report	
by Hanson.....	316
by Paul.....	274
Nominating committee.....	180
report by Veitch.....	111
Obituary	
on Charles Dayton Woods, by Bartlett.....	No. 3, iii
on Charles Lyndall Penny, by Hanson.....	No. 4, iii
on Edwin Fremont Ladd, by Wiley.....	No. 1, iii
on Guilford L. Spencer, by Wiley.....	No. 2, iii
Officers, committees, referees, and associate referees for year ending October, 1926	1
Oils and fats	
changes in methods.....	44
recommendations	
by Committee C.....	81
by Jamieson.....	253
report by Jamieson.....	247
Organic and ammoniacal nitrogen, eggs and egg products, method.....	57
Paine, report, sugars and sugar products.....	154
Paint, report of committee to consider advisability of studying methods of analyzing, by Hand.....	107



	PAGE
Palkin, report, santonin	326
Palmer, report on methods for determination of total solids in liquid eggs and powdered eggs	354
Papain, report by Paul	277
Paprika oil, iodine number of, paper by Mitchell	477
Pastes, alimentary, recommendations by Committee C	92
Patten, report, inorganic plant constituents	193
Patterson and Hartwell, report of board of governors of Crop Protection Institute of National Research Council	97
Paul, report on drugs	266
Penny, Charles Lyndall, obituary by Hanson	No. 4, iii
Pepper shells in pepper, detection and determination, paper by Smith, Alfred and Mitchell	333
Pharmaceutical research, report of National Conference, by Emery	24
Phenobarbital, method for determination	51
Phenobarbital and barbital, recommendations by Committee B	79
Phenolphthalein	
in tablets, changes in methods	53
recommendations by Committee B	78
report by Paul	277
Phenylcinchoninic acid, report by Paul	277
Phosphoric acid	
gravimetric determination, report by Ross	182
in fertilizers, recommendations by Committee A	74
Phosphorus and sulfur in the seed of plants, report by Latschaw	194
Plant constituents, inorganic, report by Patten	193
Plants	
inorganic constituents, recommendations by Committee A	75
sulfur and phosphorus content of seed, report by Latschaw	194
Polariscopic methods	
recommendations	
by Committee A	73
by Zerban	177
report by Zerban	166
Polysaccharides, determination of starch in presence of, report by Coe	147
Potash	
in fertilizers, recommendations by Committee A	74
in mixed fertilizers, method for determination, paper by Fraps	192
recommendations by Kerr	192
report by Kerr	191
Preparation of sample	
alimentary pastes	43
bread	42
eggs and egg products	56
Preservatives, food	
recommendations	
by Committee C	84
by Randall	361
report by Randall	358
President's address, by Browne	15
Prince, report, <del>nitrogen</del>	187
Protein	
in alimentary pastes, method for determination	44
in bread, method for determination	42
-nitrogen, water-soluble, precipitable by 40 per cent alcohol, method for determination	40
Publications, financial report October 1, 1924 to October 15, 1925, by Balcom	62
Purposes and aims of agricultural chemical analysis, address by President Browne	1
Pyramidon	
changes in methods	53
recommendations by Committee B	78
report	
by Paul	273
by Rabak	309

Quantitative determination of unsaponifiable matter in wheat flour, alimentary pastes, and eggs, paper by Hertwig and Bailey.....	122
Quinine and strychnine, separation.....	53
recommendations	
by Committee B.....	78
by Elliott.....	312
report	
by Elliott.....	310
by Paul.....	274
Rabak, report, pyramidon.....	309
Radio activity in drugs and water	
recommendations by Committee B.....	77
report by Paul.....	272
Raffinose and sucrose in sugars and sugar products, method for determination....	33
Randall, report on food preservatives.....	358
Reaction value of soils	
recommendations	
by Burgess.....	141
by Committee A.....	71
report by Burgess.....	138
Reagents, chemical, testing	
recommendations by Committee B.....	75
report by Spencer.....	347
Recommendations of referees, report of Committee C, by Doolittle.....	79
Redfield, discussion of methods for eggs.....	352
Referees	
associate referees, officers, and committees for year ending October, 1926....	1
report	
of committee A on recommendations, by Ross.....	70
of committee B on recommendations, by Lythgoe.....	75
of committee C on recommendations, by Doolittle.....	79
on recommendations, by Doolittle.....	69
Refractometric, drying, and densimetric methods for sugar-house products	
recommendations by Committee A.....	73
report by Schneller.....	156
Resolutions, report of committee, by Fraps.....	112
Ross, report	
gravimetric determination of phosphoric acid.....	182
of Committee A on recommendations of referees.....	70
Runkel, report on methods for sampling flour.....	423
Sale, report on flavors and non-alcoholic beverages.....	446
Sale and Badger, paper, method for determination of acidity of highly colored fruit-type products.....	342
Salt	
in feeding stuffs, method for determination.....	32
waters and brine	
changes in methods.....	29
recommendations	
by Badger.....	126
by Committee A.....	70
report by Badger.....	125
Sampling, report of Committee, by Blanck.....	107
Sampling flour	
recommendation by Runkel.....	426
report by Runkel.....	423
Santonin	
recommendations by Committee B.....	79
report	
by Palkin.....	326
by Paul.....	275
Schneller, report, drying, densimetric and refractometric methods for sugar-house products.....	156
Schwartz, report, bio-assay of drugs.....	330

	PAGE
Secretary-treasurer	
financial report, October 1, 1924 to October 15, 1925 (Skinner).....	64
report by Skinner.....	98
Seed of plants, sulfur and phosphorus content, report by Latahaw.....	194
Separation	
of formic acid in food products by distillation with xylene, paper by Morton and Spencer.....	221
of quinine and strychnine	
recommendations	
by Committee B.....	78
by Elliott.....	312
report by Elliott.....	310
Shaw, report, liming materials.....	141
Silver, total, method for determination.....	54
Silver compounds, ionizable, method for detection and estimation.....	55
Silver proteinate	
changes in methods.....	54
recommendations	
by Committee B.....	78
by Hitchcock.....	315
report	
by Hitchcock.....	312
by Paul.....	274
Skinner, report	
financial, October 1, 1924 to October 15, 1925.....	64
of committee on bibliography.....	110
of secretary-treasurer.....	98
Smith, Alfend, and Mitchell, paper, detection of added pepper shells in pepper...	333
Sodium cacodylate, method for determination of arsenic content.....	51, 287
Soil analysis, report of committee on revision of methods, by MacIntire.....	69
Soils, reaction value	
recommendations	
by Burgess.....	141
by Committee A.....	71
report by Burgess.....	138
Soils and liming materials, recommendations by Committee A.....	71
Specific gravity and alcohol	
recommendations	
by Committee B.....	75
by Hann.....	440
report by Hann.....	437
Spencer, Guilford L., obituary by Wiley.....	No. 2, iii
Spencer, G. S., report	
on chemical reagents.....	347
on mercurials.....	307
on moisture in flour.....	404
Spencer and Morton, paper, separation of formic acid in food products by distillation with xylene.....	221
Spices and condiments, recommendations by Committee B.....	75
Starch	
in feeding stuffs, method for determination.....	31
in linseed meal, determination in presence of interfering polysaccharides...	72, 147
Starch conversion products, recommendations by Committee A.....	72
Sterling, report, method for determination of moisture by distillation with toluene	153
Stock feed adulteration	
recommendations	
by Committee A.....	72
by Gensler.....	153
report by Gensler.....	148
Strychnine, determination in poisoned grains, paper by Elmore.....	224

	PAGE
Strychnine and quinine	
method for separation.....	53
recommendation	
by Committee B.....	78
by Elliott.....	312
report	
by Elliott.....	310
by Paul.....	274
Sucrose and raffinose in sugars and sugar products, method for determination....	33
Sugar, determination in meat products, recommendations by Committee C.....	88
Sugar-house products, report on drying, densimetric, and refractometric methods	
by Schneller.....	156
Sugars	
chemical methods for reducing	
recommendations	
by Committee A.....	73
by Jackson.....	178
report by Jackson.....	178
polariscopic methods	
recommendations	
by Committee A.....	73
by Zerban.....	177
report by Zerban.....	166
Sugars and sugar products	
changes in methods.....	33
recommendations by Committee A.....	72
report by Paine.....	154
Sulfur and phosphorus in the seed of plants, report by Latashaw.....	194
Sulfuric and nitric acid solution, determination of electrolytic deposition.....	38
Tanning materials and leather, report of referee.....	127
Testing chemical reagents	
recommendations by Committee B.....	75
report by Spencer.....	347
Toluene, distillation with, for determinatoin of moisture	
recommendations by Sterling.....	154
report by Sterling.....	153
Tonics, bitter, and laxatives	
recommendations by Committee B.....	77
report by Fuller.....	306
Total solids, eggs and egg products, methods.....	56
Treasurer, report by Skinner.....	64, 98
Turpentine	
report	
by Grotlisch.....	473
by Paul.....	277
Turpentine oil (spirits of turpentine)	
changes in methods.....	55
recommendations by Committee B.....	75
United States Pharmacopeia, revision, report of committee, by Kebler.....	95
Unsaponifiable matter in wheat flour, alimentary pastes, and eggs, quantitative	
determination, paper by Hertwig and Bailey.....	122
Unsaponifiable residue in fats and oils, method for determination.....	45
Vacuum method for total solids, eggs and egg products.....	56
Vanilla resins in vanilla, method for determination.....	48
Veitch, report, nominating committee.....	111
Veronal and luminal, report by Paul.....	276
Vinegars	
changes in methods.....	46
recommendations	
by Clarke.....	445
by Committee C.....	86
report by Clarke.....	440

	PAGE
Visitors and members present, 1935 meeting.....	6
Wales, paper, note on the diethylphthalate test.....	476
Warren, report, chaulmoogra oil.....	290
Waterman, paper, casein in milk.....	246
Waters, brine and salt changes in methods.....	29
recommendations	
by Badger.....	126
by Committee A.....	70
report by Badger.....	125
Wheat flour, alimentary pastes, and eggs, quantitative determination of unsaponi- fiable matter, paper by Hertwig and Bailey.....	122
Wheat flour, glutenin content	
recommendation by Blish.....	423
report by Blish.....	417
Wichmann method for determination of lead number of flavoring extracts.....	47
Wiley, Honorary President,	
address.....	382
obituary on	
Edwin Fremont Ladd.....	No. 1, iii
Guilford L. Spencer.....	No. 2, iii
Woods, Charles Dayton, obituary by Bartlett.....	No. 3, iii
Xylene, use in distillation for separation of formic acid in food products, paper by Morton and Spencer.....	221
Zerban, report, polariscopic methods.....	166





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